

## PATENT COOPERATION TREATY

**PCT**

**COMMUNICATION IN CASES FOR WHICH  
NO OTHER FORM IS APPLICABLE**

From the INTERNATIONAL BUREAU

To:

SHEINBEIN, Sol  
c/o Castorina, Anthony  
2001 Jefferson Davis Highway  
Suite 207  
Arlington, VA 22202  
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year) 23 May 2001 (23.05.01)	
Applicant's or agent's file reference 74/79	<b>REPLY DUE</b> see paragraph 1 below
International application No. PCT/US99/26094	International filing date (day/month/year) 04 November 1999 (04.11.99)
Applicant TECHNION RESEARCH & DEVELOPMENT FOUNDATION LTD.	

1. ☐ REPLY DUE within \_\_\_\_\_ months/days from the above date of mailing
- ☐ NO REPLY DUE, however, see below
- ☒ IMPORTANT COMMUNICATION
- ☐ INFORMATION ONLY

## 2. COMMUNICATION:

The International Bureau regrets to inform the applicant that, due to late receipt of the record copy, the above identified international application has not been published promptly after the expiration of 18 months from the priority date, as provided in PCT Article 21(2)(a).

International publication will now take place on 21 June 2001 (21.06.01).

Meanwhile, the International Bureau will communicate a copy of the international application to each designated Office, in accordance with PCT Article 20.

A copy of this notification has been sent to the receiving Office RO/US and all designated Offices.

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Fiona DOHERTY
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

## PATENT COOPERATION TREATY

PCT

COMMUNICATION OF  
INTERNATIONAL APPLICATIONS

(PCT Article 20)

From the INTERNATIONAL BUREAU

To:

Commissioner  
US Department of Commerce  
United States Patent and Trademark  
Office, PCT  
2011 South Clark Place Room  
CP2/5C24  
Arlington, VA 22202  
ETATS-UNIS D'AMERIQUE  
in its capacity as designated Office

Date of mailing:

23 May 2001 (23.05.01)

The International Bureau transmits herewith copies of the international applications having the following international application numbers and international publication numbers:

International application no.:

PCT/US99/26094

International publication no.:

The International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer:

J. Zahra

Telephone No.: (41-22) 338.83.38

# PATENT COOPERATION TREATY

00/20184

From the INTERNATIONAL SEARCHING AUTHORITY

To: MARK M. FREIDMAN  
C/O ANTHONY CASTORINA  
2001 JEFFERSON DAVIS HIGHWAY, SUITE 207  
ARLINGTON, VA 22202

## PCT

### NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference 74/79	Date of Mailing (day/month/year) <b>11 JUL 2000</b> <sup>d</sup> 11 Sep 2000
International application No. PCT/US99/26094	International filing date (day/month/year) 28 FEBRUARY 2000
Applicant TECHNION RESEARCH & DEVELOPMENT FOUNDATION LTD.	

1. ☒ The applicant is hereby notified that the international search report has been established and is transmitted herewith.

**Filing of amendments and statement under Article 19:**  
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

**When?** The time limit for filing such amendments is normally 2 months from the date of transmittal of the international search report; however, for more details, see the notes on the accompanying sheet.

**Where?** Directly to the International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland  
Facsimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in rules 90 bis 1 and 90 bis 3, respectively, before the completion of the technical preparations for international publication.

Within 19 months from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within 20 months from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231  Facsimile No. (703) 305-3230	Authorized officer <i>Jayne Bruders</i> ANNE MARIE S. BECKERLEG  Telephone No. (703) 308-0196
---	--

# PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: MARK M. FREIDMAN  
C/O ANTHONY CASTORINA  
2001 JEFFERSON DAVIS HIGHWAY, SUITE 207  
ARLINGTON, VA 22202

## PCT

### NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

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International application No. PCT/US99/26094	International filing date (day/month/year) 28 FEBRUARY 2000
Applicant TECHNION RESEARCH & DEVELOPMENT FOUNDATION LTD.	

1. ☒ The applicant is hereby notified that the international search report has been established and is transmitted herewith.

**Filing of amendments and statement under Article 19:**  
The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

**When?** The time limit for filing such amendments is normally 2 months from the date of transmittal of the international search report; however, for more details, see the notes on the accompanying sheet.

**Where?** Directly to the International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland  
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For more detailed instructions, see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

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☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in rules 90 *bis* 1 and 90 *bis* 3, respectively, before the completion of the technical preparations for international publication.

Within 19 months from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within 20 months from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231  Facsimile No. (703) 305-3230	Authorized officer ANNE MARIE S. BECKERLEG  Telephone No. (703) 308-0196
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## PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 74/79	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/US99/26094	International filing date (day/month/year) 28 FEBRUARY 2000	(Earliest) Priority Date (day/month/year) 27 NOVEMBER 1998
Applicant TECHNION RESEARCH & DEVELOPMENT FOUNDATION LTD.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

## 1. Basis of the report

a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

2. ☐ Certain claims were found unsearchable (See Box I).

3. ☐ Unity of invention is lacking (See Box II).

4. With regard to the title,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the abstract,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No. \_\_\_\_\_

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/26094**A. CLASSIFICATION OF SUBJECT MATTER**IPC(7) : A61K 48/00, 39/395; C07K 16/00  
US CL : 514/44; 530/387.1; 424/93.2, 130.1

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44; 530/387.1; 424/93.2, 130.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	OKAMURA, H. et al. Cloning of a new cytokine that induces INF- $\gamma$ production by T cells. Nature. 02 November 1995, Vol. 378, pages 88-91, especially page 88.	1-2,4 ----- 3, 5-11
X --- Y	TANIGUCHI, M. et al. Characterization of anti-human interleukin-18/interferon inducing factor (IGIF) monoclonal antibodies and their application in the measurement of human IL-18 by ELISA. J. Immunol. Meth. 1997, Vol. 206, No. 1-2, pages 107-113, especially page 107	1-3 ----- 4-11
Y	Database BIOSIS on STN. REVOLTELLA. R.P. 'Natural and therapeutically-induced antibodies to cytokines'. Biotherapy. 1998, Vol. 10. No. 4. pages 321-331, abstract.	12-13

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"B" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 19 JUNE 2000	Date of mailing of the international search report 11 JUL 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer <i>Jayne Bridger</i> ANNE MARIE S. BECKERLEG Telephone No. (703) 308-0196

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/26094

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KIM. J.J. et al. Modulation of amplitude and direction of in vivo immune responses by co-administration of cytokine gene expression cassettes with DNA immunogens. Eur. J. Immunol. March 1998, Vol. 28, No. 3, pages 1089-1103, especially page 1089.	14-34

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US99/26094

**B. FIELDS SEARCHED**

Electronic data bases consulted (Name of data base and where practicable terms used):

DIALOG-Medline, Embase, Cancerlit, Scisearch, Biosis, BRS-EAST-USPatfull, Derwint, EPO

search terms: interleukin 18 or il-18 or interferon gamma inducing factor or igif and antibod? or nucleic acid? or vector?  
or plasmid?



# PATENT COOPERATION TREATY

TB

## PCT

### INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

REC'D 13 JUL 2000

Applicant's or agent's file reference 74/79	<div style="display: flex; justify-content: space-between;"> <div style="width: 40%;">FOR FURTHER ACTION</div> <div style="width: 60%;">see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.</div> </div>	
International application No. PCT/US99/26094	International filing date (day/month/year) 28 FEBRUARY 2000	(Earliest) Priority Date (day/month/year) 27 NOVEMBER 1998
Applicant TECHNION RESEARCH & DEVELOPMENT FOUNDATION LTD.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

**1. Basis of the report**

a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

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6. The figure of the drawings to be published with the abstract is Figure No. \_\_\_\_\_

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/26094

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(7) : A61K 48/00, 39/395; C07K 16/00

US CL : 514/44; 530/387.1; 424/93.2, 130.1

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44; 530/387.1; 424/93.2, 130.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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X --- Y	OKAMURA, H. et al. Cloning of a new cytokine that induces INF- $\gamma$ production by T cells. Nature. 02 November 1995, Vol. 378, pages 88-91, especially page 88.	1-2,4 ----- 3, 5-11
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☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
*A* document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
*B* earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
*L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

19 JUNE 2000

Date of mailing of the international search report

11 JUL 2000

Name and mailing address of the ISA/US  
Commissioner of Patents and Trademarks  
Box PCT  
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

ANNE MARIE S. BECKERLEG

Telephone No. (703) 308-0196

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/26094

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KIM. J.J. et al. Modulation of amplitude and direction of in vivo immune responses by co-administration of cytokine gene expression cassettes with DNA immunogens. Eur. J. Immunol. March 1998, Vol. 28, No. 3, pages 1089-1103, especially page 1089.	14-34

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/26094

## B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

DIALOG-Medline, Embase, Cancerlit, Scisearch, Biosis, BRS-EAST-USPatfull, Derwint, EPO  
search terms: interleukin 18 or il-18 or interferon gamma inducing factor or igif and antibod? or nucleic acid? or vector?  
or plasmid?

## PCT

REC'D 24 SEP 2001

WIPO

PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 74/79	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/US99/26094	International filing date (day/month/year) 04 NOVEMBER 1999	Priority date (day/month/year) 27 NOVEMBER 1998
International Patent Classification (IPC) or national classification and IPC IPC(7): A61K 48/00, 39/395; C07K 16/00 and US Cl.: 514/44; 530/387.1; 424/93.2, 130.1		
Applicant TECHNION RESEARCH & DEVELOPMENT FOUNDATION LTD.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of <u>5</u> sheets. <input type="checkbox"/> This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT). These annexes consist of a total of <u>0</u> sheets.
3. This report contains indications relating to the following items:  I <input checked="" type="checkbox"/> Basis of the report II <input type="checkbox"/> Priority III <input type="checkbox"/> Non-establishment of report with regard to novelty, inventive step or industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability, citations and explanations supporting such statement VI <input type="checkbox"/> Certain documents cited VII <input type="checkbox"/> Certain defects in the international application VIII <input checked="" type="checkbox"/> Certain observations on the international application

Date of submission of the demand 22 JUNE 2000	Date of completion of this report 10 SEPTEMBER 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer <i>Anne Marie Beckerleg</i> ANNE MARIE BECKERLEG Telephone No. (703) 308-0196

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US99/26094

**I. Basis of the report**

## 1. With regard to the elements of the international application:\*

☒ the international application as originally filed☒ the description:pages 1-30, as originally filedpages NONE, filed with the demandpages NONE, filed with the letter of \_\_\_\_\_☒ the claims:pages 31-34, as originally filedpages NONE, as amended (together with any statement) under Article 19pages NONE, filed with the demandpages NONE, filed with the letter of \_\_\_\_\_☒ the drawings:pages 1-6, as originally filedpages NONE, filed with the demandpages NONE, filed with the letter of \_\_\_\_\_☒ the sequence listing part of the description:pages 1-2, as originally filedpages NONE, filed with the demandpages NONE, filed with the letter of \_\_\_\_\_

## 2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language \_\_\_\_\_ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).☐ the language of publication of the international application (under Rule 48.3(b)).☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

## 3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☒ contained in the international application in printed form.☐ filed together with the international application in computer readable form.☐ furnished subsequently to this Authority in written form.☐ furnished subsequently to this Authority in computer readable form.☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.4. ☒ The amendments have resulted in the cancellation of:☒ the description, pages NONE☒ the claims, Nos. NONE☒ the drawings, sheets/fig NONE5. ☐ This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).\*\*

\* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

\*\*Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/US99/26094

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement****1. statement**

Novelty (N)	Claims <u>5-34</u>	YES
	Claims <u>1-4</u>	NO
Inventive Step (IS)	Claims <u>5-34</u>	YES
	Claims <u>1-4</u>	NO
Industrial Applicability (IA)	Claims <u>1-34</u>	YES
	Claims <u>NONE</u>	NO

**2. citations and explanations (Rule 70.7)**

Claims 1-4 lack novelty under PCT Article 33(2) and inventive step under PCT Article 33(3) as being anticipated by and obvious over Taniguchi et al. (J. Immunol. Meth., 1997, Vol. 206, pages 107-113).

The claims recite an antibody comprising an immunoglobulin capable of binding an interferon gamma inducing factor. The claims further recite wherein the antibody is polyclonal or monoclonal, or wherein the antibody is a neutralizing antibody to said interferon gamma inducing factor in affecting cells to produce interferon gamma.

Taniguchi et al. teaches the generation of polyclonal and monoclonal antibodies against IL-18/interferon-gamma-inducing factor (Taniguchi et al., page 108, sections 2.2 and 2.3). Taniguchi et al. further teaches anti-IL-18 monoclonal antibodies which are capable of neutralizing the bioactivity of IL-18 in inducing interferon gamma (Taniguchi et al., page 110, Table 1, and page 11, Figure 1). Thus, by teaching all the elements of the claims, Taniguchi et al. anticipates the instant invention.

Claims 5-34 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest the treatment of multiple sclerosis by administering any of the following: IL-18 protein, a gene encoding IL-18, cells expressing IL-18, or antibodies to IL-18.

Claims 1-34 meet the criteria set out in PCT Article 33(4) for industrial applicability.

\_\_\_\_\_ NEW CITATIONS \_\_\_\_\_

NONE

**VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 6-9, 12, and 14-27 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims are indefinite for the following reason(s): the claims recite a method for treating a mammal for inducing protective immunity against multiple sclerosis. The syntax of the claims is confusing as it is unclear whether the method is for treating multiple sclerosis or for inducing protective immunity against multiple sclerosis. In light of the specification, it is suggested that the claim language be amended to recite, "A method for inducing protective immunity against multiple sclerosis..."

The description is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 5 because it fails to adequately enable practice of the claimed invention for the following reasons. The instant invention is drawn to the treatment of multiple sclerosis by generating protective immunity. The description discloses that protective immunity in the form of autoantibodies against interferon gamma inducing factor/IL-18 can be generated by administering a recombinant vector encoding IL-18, cells modified to express IL-18, or the IL-18 protein itself.

The description does not provide an enabling disclosure for generating protective immunity against multiple sclerosis by administering any and all vectors encoding IL-18 under transcriptional control of any promoter using any and all routes of administration or for the administration of IL-18 protein. The description's working examples are limited to the prevention of active and transferred EAE in rats by administration of anti-IL-18 antibody. The description does not provide sufficient guidance for vector/promoter combinations which result in the production of sufficient levels of IL-18 protein such that sufficient titers of anti-IL-18 antibodies are produced in a patient to protect against disease symptoms. At the time of filing, numerous factors were recognized which complicate the gene therapy art and which have not been shown to be overcome by routine experimentation, including the fate of the DNA vectors in terms of volume of distribution and rate of clearance in various tissues, the fraction of vector taken up by the target cell population, the rate of DNA degradation, the level and stability of mRNA produced, and the level and stability of the protein produced. The description reads on the direct in vivo administration of recombinant constructs encoding IL-18 and on the ex vivo administration of autologous cells transfected/transduced /infected with the recombinant construct. Recombinant constructs known at the time of filing include adenoviral, retroviral, vaccinia virus vectors, herpes viral vectors, and DNA plasmids. The description does not provide sufficient guidance as to the (Continued on Supplemental Sheet.)



**Supplemental B x**

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

Sheet 10

**VIII. CERTAIN OBSERVATIONS ON THE APPLICATION (Continued):**

intravenous, subcutaneous, or oral administration of cytokine encoding vectors, or demonstrate that the intravenous or intraperitoneal administration of a plasmid encoding IL-18 would produce titers of autoantibodies capable of protecting against EAE. The description also does not provide guidance for making recombinant constructs other than recombinant DNA plasmids or provide guidance as to the dosage, routes of administration, or level of expression of cytokine necessary to generate protective immunity against an autoimmune disease. Further, the description does not provide guidance as to the properties or characteristics of autologous cells capable of stimulating autoantibody generation to an expressed cytokine in vivo, or provide guidance as to the dosage or route of administration of transduced/transfected cytokine expressing cells capable of generating levels of anti-cytokine autoantibodies sufficient to protect against autoimmune disease.

The description also fails to provide an enabling disclosure for the treatment of multiple sclerosis by administering IL-18 protein. While the description provides guidance as to the level of anti-IL-18 antibody which correlates to a protective effect in EAE rats, the description does not provide any guidance as to the level of IL-18 protein that correlates to the generation of therapeutic levels of anti-IL-18 antibodies in any host. Further, the description fails to provide sufficient guidance for routes of protein administration that result in the production of neutralizing levels of anti-IL-18. At the time of filing, it was known that the therapeutic potential of any compound in vivo is significantly affected by the physiological conditions at the site of administration, i.e. oral versus subcutaneous, the rate of clearance of the compound, i.e. subcutaneous versus intravenous, and the half-life and stability of the compound under physiological conditions. The specification does not provide any guidance as to any of these aspects of therapeutic gene or protein delivery. It is further noted that the description teaches that the activity of IL-18 is associated with the development of Th1 type cells which have been linked to disease manifestation. In the absence of any direct teachings or evidence in the form of working examples, it is unpredictable whether the generation of any level of antibody against IL-18 would have any effect on multiple sclerosis in the presence of exogenously delivered IL-18 protein, or in the presence of cells continuously expressing IL-18.

Thus, due to the art recognized unpredictability of achieving therapeutic levels of gene expression using nucleic acid vectors, the breadth of the claims, and the lack of guidance provided by the description for the parameters affecting delivery and expression of the IL-18 gene or the IL-18 protein at a sufficient level and length of time to generate protective immunity against multiple sclerosis, it would have required undue experimentation to practice the invention as claimed.

Claims 1-34 are objected to as lacking clarity under PCT Rule 66.2(a)(v) because practice of the claimed invention is not enabled as required under PCT Rule 5.1(a) for the reasons set forth in the immediately preceding paragraph.

RECORD COPY

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only	
International Application No.	PCT/US 99/26094
International Filing Date	28 FEB 2000
PCT INTERNATIONAL APPLICATION RO/US	
Name of receiving Office and "PCT International Application"	
Applicant's or agent's file reference (if desired) (12 characters maximum) 74/79	

24 NOV 99  
(24.01.99)

As per #23

Box No. I	TITLE OF INVENTION	
	INTERFERON GAMMA INDUCING FACTOR BASED VACCINE AND USE OF SAME FOR PROTECTIVE IMMUNITY AGAINST MULTIPLE SCLEROSIS	
Box No. II	APPLICANT	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)		<input type="checkbox"/> This person is also inventor.
Technion Research & Development Foundation Ltd. Senate House Technion City Haifa 32000 Israel		Telephone No.
		Facsimile No.
		Teleprinter No.
State (that is, country) of nationality: IL		State (that is, country) of residence: IL
This person is applicant for the purposes of: <input type="checkbox"/> all designated States <input checked="" type="checkbox"/> all designated States except the United States of America <input type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box		
Box No. III	FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)	
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)		This person is:
CARIN, Nathan 9 Capri St. Haifa.34384 Israel		<input type="checkbox"/> applicant only
		<input checked="" type="checkbox"/> applicant and inventor
		<input type="checkbox"/> inventor only (If this check-box is marked, do not fill in below.)
State (that is, country) of nationality: IL		State (that is, country) of residence: IL
This person is applicant for the purposes of: <input type="checkbox"/> all designated States <input type="checkbox"/> all designated States except the United States of America <input checked="" type="checkbox"/> the United States of America only <input type="checkbox"/> the States indicated in the Supplemental Box		
<input checked="" type="checkbox"/> Further applicants and/or (further) inventors are indicated on a continuation sheet.		
Box No. IV	AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE	
The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:		<input type="checkbox"/> agent <input checked="" type="checkbox"/> common representative
Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)		Telephone No.
FRIEDMAN, Mark M. c/o CASTORINA, Anthony 2001 Jefferson Davis Highway, Suite 207 Arlington, Virginia 22202 U.S.		703-4151581
		Facsimile No.
		703-4154864
		Teleprinter No.
<input type="checkbox"/> Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.		

## Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

*If none of the following sub-boxes is used, this sheet should not be included in the request.*

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

WILDBAUM, Gizi  
3 Mairon St.  
Miriati-Yam 29036  
Israel

This person is:

- ☐ applicant only  
☒ applicant and inventor  
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

IL

State (that is, country) of residence:

IL

This person is applicant for the purposes of:

☐ all designated States

☐ all designated States except the United States of America

☒ the United States of America only

☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

FRIEDMAN, Mark M.  
1 Alharizi  
Raanana 43406  
Israel

This person is:

- ☒ applicant only  
☐ applicant and inventor  
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

US

State (that is, country) of residence:

IL

This person is applicant for the purposes of:

☐ all designated States

☐ all designated States except the United States of America

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Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

This person is:

- ☐ applicant only  
☐ applicant and inventor  
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant for the purposes of:

☐ all designated States

☐ all designated States except the United States of America

☐ the United States of America only

☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

This person is:

- ☐ applicant only  
☐ applicant and inventor  
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant for the purposes of:

☐ all designated States

☐ all designated States except the United States of America

☐ the United States of America only

☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

**Supplemental Box**

If the Supplemental Box is not used, this sheet should not be included in the request.

1. If, in any of the Boxes, **the space is insufficient** to furnish all the information: in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:

- (i) **if more than two persons are involved as applicants and/or inventors** and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;
- (ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication **"the States indicated in the Supplemental Box"** is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;
- (iii) if, in Box No. II or in any of the sub-boxes of Box No. III, **the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America**: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;
- (iv) if, in addition to the agent(s) indicated in Box No. IV, there are **further agents**: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;
- (v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication **"patent of addition,"** or **"certificate of addition,"** or if, in Box No. V, the name of the United States of America is accompanied by an indication **"continuation"** or **"continuation-in-part"**: in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;
- (vi) if, in Box No. VI, there are **more than three earlier applications whose priority is claimed**: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;
- (vii) if, in Box No. VI, **the earlier application is an ARIPO application**: in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed.

2. If, with regard to the **precautionary designation statement** contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.

3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning **non-prejudicial disclosures or exceptions to lack of novelty**: in such case, write "Statement concerning non-prejudicial disclosures or exceptions to lack of novelty" and furnish that statement below.

Continuation of Box III:

Mark M. Friedman is applicant  
for the purpose of TAJIKISTAN only.

28 FEB 2000

## Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) (mark the applicable check-boxes; at least one must be marked):

## Regional Patent

- ☒ AP ARIPO Patent: GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SL Sierra Leone, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ EA Eurasian Patent: AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ EP European Patent: AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ OA OAPI Patent: BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line) .....

National Patent (if other kind of protection or treatment desired, specify on dotted line):

- |  |  |
|--|--|
| <input checked="" type="checkbox"/> AE United Arab Emirates                  | <input checked="" type="checkbox"/> LR Liberia   |
| <input checked="" type="checkbox"/> AL Albania                               | <input checked="" type="checkbox"/> LS Lesotho   |
| <input checked="" type="checkbox"/> AM Armenia                               | <input checked="" type="checkbox"/> LT Lithuania   |
| <input checked="" type="checkbox"/> AT Austria                               | <input checked="" type="checkbox"/> LU Luxembourg  |
| <input checked="" type="checkbox"/> AU Australia                             | <input checked="" type="checkbox"/> LV Latvia  |
| <input checked="" type="checkbox"/> AZ Azerbaijan                            | <input checked="" type="checkbox"/> MD Republic of Moldova   |
| <input checked="" type="checkbox"/> BA Bosnia and Herzegovina                | <input checked="" type="checkbox"/> MG Madagascar  |
| <input checked="" type="checkbox"/> BB Barbados                              | <input checked="" type="checkbox"/> MK The former Yugoslav Republic of Macedonia                             |
| <input checked="" type="checkbox"/> BG Bulgaria                              |  |
| <input checked="" type="checkbox"/> BR Brazil                                | <input checked="" type="checkbox"/> MN Mongolia  |
| <input checked="" type="checkbox"/> BY Belarus                               | <input checked="" type="checkbox"/> MW Malawi  |
| <input checked="" type="checkbox"/> CA Canada                                | <input checked="" type="checkbox"/> MX Mexico  |
| <input checked="" type="checkbox"/> CH and LI Switzerland and Liechtenstein  | <input checked="" type="checkbox"/> NO Norway  |
| <input checked="" type="checkbox"/> CN China                                 | <input checked="" type="checkbox"/> NZ New Zealand   |
| <input checked="" type="checkbox"/> CU Cuba                                  | <input checked="" type="checkbox"/> PL Poland  |
| <input checked="" type="checkbox"/> CZ Czech Republic                        | <input checked="" type="checkbox"/> PT Portugal  |
| <input checked="" type="checkbox"/> DE Germany                               | <input checked="" type="checkbox"/> RO Romania   |
| <input checked="" type="checkbox"/> DK Denmark                               | <input checked="" type="checkbox"/> RU Russian Federation  |
| <input checked="" type="checkbox"/> EE Estonia                               | <input checked="" type="checkbox"/> SD Sudan   |
| <input checked="" type="checkbox"/> ES Spain                                 | <input checked="" type="checkbox"/> SE Sweden  |
| <input checked="" type="checkbox"/> FI Finland                               | <input checked="" type="checkbox"/> SG Singapore   |
| <input checked="" type="checkbox"/> GB United Kingdom                        | <input checked="" type="checkbox"/> SI Slovenia  |
| <input checked="" type="checkbox"/> GD Grenada                               | <input checked="" type="checkbox"/> SK Slovakia  |
| <input checked="" type="checkbox"/> GE Georgia                               | <input checked="" type="checkbox"/> SL Sierra Leone  |
| <input checked="" type="checkbox"/> GH Ghana                                 | <input checked="" type="checkbox"/> TJ Tajikistan  |
| <input checked="" type="checkbox"/> GM Gambia                                | <input checked="" type="checkbox"/> TM Turkmenistan  |
| <input checked="" type="checkbox"/> HR Croatia                               | <input checked="" type="checkbox"/> TR Turkey  |
| <input checked="" type="checkbox"/> HU Hungary                               | <input checked="" type="checkbox"/> TT Trinidad and Tobago   |
| <input checked="" type="checkbox"/> ID Indonesia                             | <input checked="" type="checkbox"/> UA Ukraine   |
| <input checked="" type="checkbox"/> IL Israel                                | <input checked="" type="checkbox"/> UG Uganda  |
| <input checked="" type="checkbox"/> IN India                                 | <input checked="" type="checkbox"/> US United States of America  |
| <input checked="" type="checkbox"/> IS Iceland                               |  |
| <input checked="" type="checkbox"/> JP Japan                                 | <input checked="" type="checkbox"/> UZ Uzbekistan  |
| <input checked="" type="checkbox"/> KE Kenya                                 | <input checked="" type="checkbox"/> VN Viet Nam  |
| <input checked="" type="checkbox"/> KG Kyrgyzstan                            | <input checked="" type="checkbox"/> YU Yugoslavia  |
| <input checked="" type="checkbox"/> KP Democratic People's Republic of Korea | <input checked="" type="checkbox"/> ZA South Africa  |
|  | <input checked="" type="checkbox"/> ZW Zimbabwe  |
| <input checked="" type="checkbox"/> KR Republic of Korea                     | Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet: |
| <input checked="" type="checkbox"/> KZ Kazakhstan                            | <input type="checkbox"/> .....   |
| <input checked="" type="checkbox"/> LC Saint Lucia                           | <input type="checkbox"/> .....   |
| <input checked="" type="checkbox"/> LK Sri Lanka                             |  |

**Precautionary Designation Statement:** In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. (Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)

<b>Box No. VI PRIORITY CLAIM</b>		<input type="checkbox"/> Further priority claims are indicated in the Supplemental Box.		
Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is:		
		national application: country	regional application:* regional Office	international application: receiving Office
item (1) (27.11.98) 27 NOV 1998	09/200,716	US		
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) (only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office) identified above as item(s): (1)

\* Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.

### Box No. VII INTERNATIONAL SEARCHING AUTHORITY

**Choice of International Searching Authority (ISA)**  
(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):

ISA / US

**Request to use results of earlier search; reference to that search** (if an earlier search has been carried out by or requested from the International Searching Authority):

Date (day/month/year)

Number

Country (or regional Office)

### Box No. VIII CHECK LIST: LANGUAGE OF FILING

This international application contains the following number of sheets:  
request : 5

description (excluding  
sequence listing part) : 30

claims : 4

abstract : 1

drawings : 6

sequence listing part  
of description : 2

**Total number of sheets : 48**

This international application is accompanied by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☐ other (specify):

**Figure of the drawings which  
should accompany the abstract:**

**Language of filing of the  
international application:** English

### Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Mark M. Friedman  
common representative

(04.11.99)

<p>1. Date of actual receipt of the purported international application: <b>520 Rec'd PCT/PTO 04 NOV 1999</b></p>		<p>2. Drawings:</p> <p><input type="checkbox"/> received:</p> <p><input type="checkbox"/> not received:</p>
<p>3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application: <b>28 FEB 2000 (28.02.00)</b></p>		
<p>4. Date of timely receipt of the required corrections under PCT Article 11(2):</p>		
<p>5. International Searching Authority (if two or more are competent): <b>ISA / US</b></p>		
<p>6. <input type="checkbox"/> Transmittal of search copy delayed until search fee is paid.</p>		

For International Bureau use only	
Date of receipt of the record copy by the International Bureau: <b>16 MAY 2000</b>	<b>( 16.05.00 )</b>

1  
INTERFERON GAMMA INDUCING FACTOR BASED VACCINE AND  
USE OF SAME FOR PROTECTIVE IMMUNITY AGAINST MULTIPLE  
SCLEROSIS

5 FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to the treatment of multiple sclerosis and, more particularly, to interferon gamma inducing factor (IGIF) based vaccines for effecting same.

Based on their cytokine profile CD4<sup>+</sup> T-cells can be divided to Th1  
10 cells that produce large amounts of interferon gamma (IFN- $\gamma$ ) and TNF- $\alpha$ ,  
and, to a much lesser extent, IL-4 and IL-10; Th2 cells that produce IL-4,  
IL-10, and IL-13 and, to a much lesser extent, IFN- $\gamma$  and TNF- $\alpha$  (1-10), and  
the newly defined Th3 cells that produce significant amounts of  
transforming growth factor beta (TGF- $\beta$ ) and have been associated with oral  
15 tolerance (11).

Th1 cells selected in response to various auto-antigens transfer T-cell  
mediated autoimmune diseases, whereas IL-4 secreting Th2 cells, selected  
in response to these same antigens, either inhibit or exert no profound effect  
on the inflammatory process (5, 12-24).

20 High levels of IFN- $\gamma$  and low levels of IL-4 positively select for Th1  
cells, whereas, low levels of IFN- $\gamma$  together with high levels of IL-4 mediate  
Th2 selection (1-6).

Interferon gamma inducing factor (IGIF, interleukin-18, IL-18) is a  
recently described cytokine (25) that shares structural features with the  
25 interleukin-1 (IL-1) family of proteins (26). Activation of IGIF is mediated  
by interleukin-1 beta converting enzyme (ICE) (27, 28). Like IL-12, IGIF  
is a potent inducer of the production of IFN- $\gamma$  by Th1 and natural killer  
(NK) cells, and acts on Th1 cells together with IL-12 in a synergistic  
manner (25, 29-32).

30 Experimental autoimmune encephalomyelitis (EAE) is a T cell  
mediated autoimmune disease of the central nervous system (CNS) which,  
for many years and for a variety of experimental protocols, serves as a  
model for the human disease, multiple sclerosis (MS), a chronic  
degenerative disease marked by patchy destruction of the myelin that  
35 surrounds and insulates nerve fibers and mild to severe neural and muscular  
impairments, since in both diseases circulating leukocytes penetrate the  
blood brain barrier and damage myelin resulting in impaired nerve  
conduction and paralysis (33, 34). Antigen specific T cells are thought to  
play a pivotal role in the manifestation of both diseases (35-37).

The role of Th1 cells in the manifestation of EAE has been widely studied. Th1 but not Th2 cells transfer the disease to normal naive recipients (18). Shifting the Th1/Th2 balance towards Th2 cells by *in vivo* administration of IL-4 (12), by antibodies to B7-1 (14), by soluble peptide therapy (38), or by administration of neutralizing antibodies to IL-12 (15) markedly suppressed EAE.

It has recently been shown that IGIF is a more potent inducer of IFN- $\gamma$  producing Th1 cells than is IL-12 and thus plays an important role in Th1 responses (25). However, the possible role of anti-IGIF immunotherapy in regulation of T cell mediated autoimmunity has never been evaluated.

While reducing the present invention to practice it has been shown, for the first time, that neutralizing antibodies to IGIF ameliorate EAE by shifting the Th1/Th2 balance towards antigen specific Th2 cells.

## SUMMARY OF THE INVENTION

The present invention disclosed the use of anti interferon gamma inducing factor antibody in the treatment of multiple sclerosis. This use can be effected in a variety of ways as further described and exemplified hereinbelow.

According to one aspect of the present invention there is provided an antibody comprising an immunoglobulin capable of binding interferon gamma inducing factor.

According to another aspect of the present invention there is provided a pharmaceutical composition for inducing protective immunity against multiple sclerosis, comprising a pharmaceutically acceptable carrier and an antibody being capable of binding an interferon gamma inducing factor.

According to still another aspect of the present invention there is provided a pharmaceutical composition for inducing protective immunity against multiple sclerosis, comprising a pharmaceutically acceptable carrier and an interferon gamma inducing factor or an immunogenic portion thereof, thereby eliciting an antibody being capable of binding the interferon gamma inducing factor *in vivo*.

According to an additional aspect of the present invention there is provided a method for treating an animal for inducing protective immunity against multiple sclerosis, the method comprising the step of administering to the animal cells being capable of producing and secreting an antibody



capable of *in vivo* neutralizing an interferon gamma inducing factor in affecting cells to produce interferon gamma.

According to yet additional aspect of the present invention there is provided a method for treating an animal for inducing protective immunity  
5 against multiple sclerosis, the method comprising the step of administering to the animal an antibody capable of *in vivo* neutralizing an interferon gamma inducing factor in affecting cells to produce interferon gamma.

According to still additional aspect of the present invention there is provided a method for treating an animal for inducing protective immunity  
10 against multiple sclerosis, the method comprising the step of administering to the animal an antigen including an interferon gamma inducing factor or an immunogenic portion thereof, thereby eliciting an antibody being capable of binding *in vivo* an interferon gamma inducing factor.

According to further features in preferred embodiments of the  
15 invention described below, the antibody is polyclonal.

According to still further features in the described preferred embodiments the antibody is monoclonal.

According to still further features in the described preferred  
20 embodiments the antibody is a neutralizing antibodies to the interferon gamma inducing factor in affecting cells to produce interferon gamma.

According to still further features in the described preferred embodiments the antibody is humanized.

According to another aspect of the present invention there is provided a method for treating an animal for inducing protective immunity  
25 against multiple sclerosis, the method comprising the step of administering to the animal a therapeutic composition including a recombinant construct including an isolated nucleic acid sequence encoding a polypeptide being capable of eliciting antibodies capable of *in vivo* neutralizing an interferon gamma inducing factor in affecting cells to produce interferon gamma.

According to yet another aspect of the present invention there is provided a method for treating an animal for inducing protective immunity  
30 against multiple sclerosis, the method comprising the steps of (a) removing cells of the animal; (b) genetically modifying the cells *in vitro* with a recombinant construct including an isolated nucleic acid sequence encoding an interferon gamma inducing factor or an immunogenic portion thereof;  
35 and (c) reintroducing the genetically modified cells to the animal.

According to still another aspect of the present invention there is provided a pharmaceutical composition for inducing protective immunity

against multiple sclerosis, comprising a pharmaceutically acceptable carrier and a recombinant construct including an isolated nucleic acid sequence encoding a polypeptide being capable of eliciting antibodies capable of *in vivo* neutralizing an interferon gamma inducing factor in affecting cells to produce interferon gamma.

According to further features in preferred embodiments of the invention described below, the nucleic acid sequence being operatively linked to one or more transcription control sequences.

According to still further features in the described preferred embodiments the transcription control sequences are selected from the group consisting of RSV control sequences, CMV control sequences, retroviral LTR sequences, SV-40 control sequences and  $\beta$ -actin control sequences.

According to still further features in the described preferred embodiments the recombinant construct is an eukaryotic expression vector.

According to still further features in the described preferred embodiments the recombinant construct is selected from the group consisting of pcDNA3, pcDNA3.1(+/-), pZeoSV2(+/-), pSecTag2, pDisplay, pEF/myc/cyto, pCMV/myc/cyto, pCR3.1, pCI, pBK-RSV, pBK-CMV, pTRES and their derivatives.

According to still further features in the described preferred embodiments the therapeutic composition is administered to the animal parenterally.

According to still further features in the described preferred embodiments the animal is a human being.

According to still further features in the described preferred embodiments the pharmaceutically acceptable carrier is selected from the group consisting of an aqueous physiologically balanced solution, an artificial lipid-containing substrate, a natural lipid-containing substrate, an oil, an ester, a glycol, a virus and metal particles.

According to still further features in the described preferred embodiments the pharmaceutically acceptable carrier comprises a delivery vehicle that delivers the nucleic acid sequences to the animal.

According to still further features in the described preferred embodiments the delivery vehicle is selected from the group consisting of liposomes, micelles, and cells.

According to still further features in the described preferred embodiments the recombinant construct is an eukaryotic expression vector.

According to still further features in the described preferred embodiments the composition is suitable for parenteral administration to a human.

The present invention successfully addresses the shortcomings of the presently known configurations by providing new horizons to the treatment of the devastating autoimmune disease multiple sclerosis.

#### BRIEF DESCRIPTION OF THE DRAWINGS

The invention herein described, by way of example only, with reference to the accompanying drawings, wherein:

FIGs. 1A-F demonstrate IGIF mRNA in the inflamed EAE brain. Figures 1A, C, G and E - Rats were injected with  $10^7$  cells from L68-86 immunized rats to allow for the development of transferred EAE. Before adoptive transfer of disease (day 0), and at various time points: before the onset of disease (day 3), at the day of onset (day 5), the peak (day 7), following recovery (day 10), and 10 days after recovery (day 20) mid-brain and brain stem samples from six different rats at each time point were examined. mRNA was isolated from each sample and subjected to RT-PCR analysis using specific oligonucleotide primers constructed for IGIF (Figure 1C) and for IFN- $\gamma$  (Figure 1E). Each amplification was calibrated to  $\beta$ -actin (Figure 1G) and verified by Southern Blot analysis. Southern blot images were objectively assessed using an FujiFilm Thermal System. Figures 1B, D, F and H - Rats were immunized with p68-86/CFA and developed active EAE. Before the induction of disease (day 0), and at various time points: before the onset of disease (day 8), at the peak (day 13), and following recovery (day 21) mid-brain and brain stem samples from six different rats at each time point were examined for mRNA transcription for IGIF (Figure 1D) and IFN- $\gamma$  (Figure 1F) and calibrated to  $\beta$ -actin (Figure 1H), as described above.

FIGs. 2A-D demonstrate that neutralizing antibodies to recombinant rat IGIF block IFN- $\gamma$  production in cultured T cells. Spleen cells from naive (Figures 2A and 2B) or from p68-86/CFA primed (day 9) Lewis rats (Figures 2C and 2D) were cultured *in vitro* with either Con A (Figures 2A and 2B), or with 100  $\mu$ M of MBP p68-86 (Figures 2C and 2D) with or without the addition of 100 ng/ml of rabbit anti-rat IGIF (IgG) neutralizing antibodies (Figures 2A and 2C) or with IgG from non-immunized rabbits (data not shown), with or without the addition of 400 ng/ml recombinant rat IGIF (Figures 2B and 2D). After 72 hours of stimulation IFN- $\gamma$  levels were

determined in the culture supernatants by an ELISA assay. Results are the mean  $\pm$  S.E. of triplicate cultures.

FIGs. 3A-B demonstrate that neutralizing antibodies to recombinant rat IGIF block the development of both active and transferred EAE. Figure 3A - Lewis rats were immunized with p68-86/CFA to induce active EAE and then separated into three groups of six rats each. Eight, ten and eleven days after induction of disease, these groups were injected IV with rabbit anti-rat IGIF (IgG fraction 100 $\mu$ g/rat), with IgG fraction purified from non-immunized rabbits (control IgG), or with PBS. The rats were then monitored daily for clinical signs of EAE by an observer blind to the treatment protocol. Results are presented as mean clinical score  $\pm$  S.E. Figure 3B - Transferred EAE was induced as described above (Figure 1). Recipients were then separated into three groups of six rats each. Three, five and seven days after induction of disease these groups were injected as described above (Figure 3A) and monitored daily for clinical signs of EAE by an observer blind to the treatment protocol. Results are presented as mean clinical score  $\pm$  S.E.

FIGs. 4A-D demonstrate alteration in IFN- $\gamma$  and IL-4 production in EAE rats injected with anti-IGIF neutralizing antibodies. Lewis rats were immunized with p68-86/CFA to induce active EAE and separated into three groups. Five and seven days after disease induction these groups were injected IV with either rabbit anti-rat IGIF (IgG fraction 100  $\mu$ g/rat), with purified IgG from non-immunized rabbits, or with PBS. Before the onset of disease (day 9) splenic T cells from three rats in each group were cultured with 100  $\mu$ M MBP p68-86 for 72 hours in stimulation medium that was not (Figures 4A and 4B) or was (Figures 4C and 4D) supplemented with recombinant rat IL-4 (5 ng/ml). After 72 hours of stimulation, IFN- $\gamma$  levels were determined in culture supernatants by an ELISA assay. Results are of triplicate cultures expressed as mean  $\pm$  S.E.

FIG. 5 demonstrates alteration in TNF- $\alpha$  production in EAE rats injected with anti-IGIF neutralizing antibodies. Levels of TNF- $\alpha$  were determined in supernatants obtained in experiment described in Figures 4A and 4B, by an ELISA assay. Results are of triplicate cultures expressed as mean  $\pm$  S.E.

## DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is of interferon gamma inducing factor (IGIF) based vaccines which can be used in the treatment of multiple sclerosis. Specifically, the present invention can be used to confer protective immunity against multiple sclerosis.

The principles and operation of the interferon gamma inducing factor (IGIF) based vaccines according to the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

In autoimmune conditions, T cells reactive to self-antigens escape elimination in the thymus, and are activated in the periphery where they can provoke damage to specific cells and organs. Perturbation of the balance between self-reactive T cells with different cytokine profiles may serve as an effective way of restraining the harmful effect of autoimmune T cells (12, 14-16, 20-22, 38, 41-43).

Cytokines present at the initiation of CD4<sup>+</sup> T cell responses determine whether a Th1 or a Th2 response will predominate (1-6). Thus, administration of IL-4 or of antibodies to IL-12 preferentially favors Th2 selection *in vivo* and thus serves as a powerful way to inhibit two different T cell mediated autoimmune diseases: EAE and IDDM (12, 15, 22)

It is well known that high levels of IFN- $\gamma$  positively select for TNF- $\alpha$  secreting Th1 cells (6). A previous study showed that administration of anti-IL-12 neutralizing antibodies blocks EAE while inducing a marked reduction of both IFN- $\gamma$  and TNF- $\alpha$  production (15). IFN- $\gamma$  and TNF- $\alpha$  together then exhibit a synergistic effect on enhancing expression of adhesion molecules on endothelial cells (44), and on eliciting the inflammatory process, which can be reversed by either anti-adhesion molecule immunotherapy (45, 46), or by blocking TNF- $\alpha$  (44, 47-50). It was previously demonstrated that EAE resistance acquired by soluble antigen therapy can be reversed by anti-IL-4 neutralizing antibodies (38).

This further demonstrated the pivotal role of the Th1/Th2 balance in regulation of T cell mediated autoimmunity (38).

IGIF is a recently described cytokine (25) that shares structural features with the interleukin-1 (IL-1) family of proteins (26). Activation of IGIF is mediated by interleukin-1 beta converting enzyme (ICE) (27, 28). Like IL-12, IGIF is a potent inducer of IFN- $\gamma$  from Th1 and NK cells, and acts on Th1 cells together with IL-12 in a synergistic manner (25, 29-32). IGIF actually has more potent IFN- $\gamma$  inducing capabilities than IL-12 and apparently utilizes a distinct signal transduction pathway for its elicitation (25, 31, 32, 51). Little is known about the role of IGIF in T cell mediated autoimmune disease. A recent study used RT PCR to demonstrate that the active stage of autoimmune diabetes in NOD mice is associated with the expression of IGIF (52).

As further detailed in the Examples section below, while conceiving and reducing the present invention to practice, specific oligonucleotide primers were used to identify and isolate interferon gamma inducing factor (IGIF) from the brain of rats with developing experimental autoimmune encephalomyelitis (EAE), a T cell mediated autoimmune disease of the central nervous system (CNS) that serves as a model for multiple sclerosis (MS).

IGIF was highly transcribed in the brain at the onset and during the course of active EAE. PCR products encoding rat IGIF were used to generate the recombinant protein which was used to induce anti-IGIF neutralizing antibodies. These antibodies significantly reduced the production of interferon gamma (IFN- $\gamma$ ) by primed T cells proliferating in response to their target myelin basic protein (MBP) epitope and by Con A activated T cells from naive donors.

When administered to rats during the development of either active or transferred EAE, these antibodies significantly blocked the development of disease.

Splenic T cells from protected rats were cultured with the encephalitogenic MBP epitope and evaluated for production of IL-4 and IFN- $\gamma$ . These cells, which proliferated, exhibited a profound increase in IL-4 production, accompanied by a significant decrease in IFN- $\gamma$  and TNF- $\alpha$  production.

An elevated expression of IGIF at the time when the secondary influx of autoimmune cells is apparent at the site of inflammation in the EAE brain (38, 39, 46, 53) is demonstrated herein for the first time. So are

neutralizing antibodies, which were generated against IGIF cloned from this site of inflammation, to block the disease by altering the *in vivo* Th1/Th2 balance in favor of Th2 selection. This alteration included a marked reduction in the production of IFN- $\gamma$ , and, most importantly, TNF- $\alpha$ , a proinflammatory cytokine that plays a critical role in T cell mediated autoimmunity (44, 47-50).

An interesting observation is that both the inhibitory effect of IGIF neutralizing antibodies and the augmentation by IGIF of IFN- $\gamma$  production are more profound on activated T cells from a naive donor than on primed T cells responding to their target epitope.

The direct role of IFN- $\gamma$  in EAE is enigmatic. Grewal et al. have used a CD40L-deficient mice that carry a transgenic T cell receptor specific for MBP to demonstrate that EAE induction is IFN- $\gamma$  dependent (54). On the other hand not only were mice lacking IFN- $\gamma$  susceptible to induction of active EAE (55) but also antibodies to IFN- $\gamma$  were found capable of enhancing this disease (56, 57). A recent study has demonstrated that IL-12 is directly involved in the generation of autoreactive Th1-cells that induce EAE, both in the presence and the absence of IFN- $\gamma$  (58). However, it could well be that alteration the Th1/Th2 balance towards IL-4 secreting Th2 cells confers EAE resistance not because it leads to a reduced production of IFN- $\gamma$ , but rather because it results in a reduced production of TNF- $\alpha$  accompanied by a marked increase in IL-4 production.

It has recently been suggested that IGIF primarily effects IFN- $\gamma$  production by Th1 but not Th2 cells (29). It is possible that immunization with p68-86/CFA induces a substantial selection of antigen specific Th2 cells, albeit not enough to inhibit the subsequent development of a Th1 mediated autoimmune disease. Hence, as shown herein, *in vivo* administration of anti IGIF neutralizing antibodies notably shift the Th1/Th2 balance in antigen specific proliferating T cells towards Th2 response.

Thus, the present invention teaches the use of an anti interferon gamma inducing factor antibody in the treatment of multiple sclerosis. This use can be effected in a variety of ways and applications, some of which are further described and exemplified hereinbelow.

According to one aspect of the present invention there is provided an antibody which comprises an immunoglobulin capable of binding interferon gamma inducing factor (IGIF, IL-18).

As used herein in the specification and in the claims section below, the terms "antibody" and "immunoglobulin", which are interchangeably used, refer to any of several classes of structurally related proteins that function as part of the immune response of an animal, which proteins include IgG, IgD, IgE, IgA, IgM and related proteins. These terms further relate to chimeric immunoglobulins which are the expression products of fused genes derived from different species. These terms further relate to immunologically active derivatives of the above proteins, including, but not limited to, an F(ab')<sub>2</sub> fragment, an Fab fragment, an Fv fragment, a heavy chain, a light chain, an unassociated mixture of a heavy chain and a light chain, a heterodimer consisting of a heavy chain and a light chain, a catalytic domain of a heavy chain, a catalytic domain of a light chain, a variable fragment of a light chain, a variable fragment of a heavy chain, and a single chain variant of the antibody. Under normal physiological conditions antibodies are found in plasma and other body fluids and in the membrane of certain cells and are produced by lymphocytes of the type denoted B cells or their functional equivalent. Antibodies of the IgG class are made up of four polypeptide chains linked together by disulfide bonds. The four chains of intact IgG molecules are two identical heavy chains referred to as H-chains and two identical light chains referred to as L-chains. The immunoglobulin or antibody according to the present invention could also be a "humanized" antibody, in which, for example animal (say murine) variable regions are fused to human constant regions, or in which murine complementarity-determining regions are grafted onto a human antibody structure (Wilder, R.B. et al., J. Clin. Oncol., 14:1383-1400, 1996). Unlike, for example, animal derived antibodies, "humanized" antibodies often do not undergo an undesirable reaction with the immune system of the subject. The terms "sFv" and "single chain antigen binding protein" refer to a type of a fragment of an immunoglobulin, an example of which is sFv CC49 (Larson, S.M. et al., Cancer, 80:2458-68, 1997). As used herein, the term "humanized antibodies" also reads on antibodies produced by non-human cells or organisms genetically modified to include nucleic acid sequences encoding a functional portion of the human immune system, wherein the resulting antibodies are substantially identical to human antibodies in that they are encoded by human derived genes. However, the term "antibody", as used herein, further relates to soluble portions of receptors capable of specifically binding their respective protein ligands, which, in that respect, function like immunoglobulins.



As used herein and in the claims, the term "animal" refers to any organism with an immune system.

According to yet another aspect of the present invention there is provided a pharmaceutical composition for inducing protective immunity against multiple sclerosis. The composition comprises a pharmaceutically acceptable carrier and an antibody being capable of binding an interferon gamma inducing factor.

Alternatively, the composition according to the present invention comprises a pharmaceutically acceptable carrier and an interferon gamma inducing factor or an immunogenic portion thereof, thereby eliciting an antibody being capable of binding the interferon gamma inducing factor *in vivo*.

As used herein the phrase "immunogenic portion" refers to an immunogenic proteinaceous compound, which may include, among optional additional components, a plurality of amino acid residues. The term "amino acid" is understood to include the 20 naturally occurring amino acid residues; those amino acid residues often modified post-translationally *in vivo*, including for example hydroxyproline, phosphoserine and phosphothreonine; and other unusual amino acid residues including, but not limited to, 2-amino adipic acid, hydroxylysine, isodesmosine, nor-valine, nor-leucine and ornithine. Furthermore, the term "amino acid" includes both D- and L-amino acid residues. The amino acid residues according to the present invention form a peptide. The latter is understood to include native peptides, including degradation products or synthetically synthesized peptides, and further to peptidomimetics, such as peptoids and semipeptoids, which are peptide analogs, which may have, for example, modifications rendering the peptides more stable or less immunogenic while contacting body fluids. Such modifications include, but are not limited to, cyclization, N terminus modification, C terminus modification, peptide bond modification, including, but not limited to, CH<sub>2</sub>-NH, CH<sub>2</sub>-S, CH<sub>2</sub>-S=O, O=C-NH, CH<sub>2</sub>-O, CH<sub>2</sub>-CH<sub>2</sub>, S=C-NH, CH=CH or CF=CH, backbone modification and residue modification. Methods for preparing peptidomimetic compounds are well known in the art and are specified in Quantitative Drug Design, C.A. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press (1992), which is incorporated by reference as if fully set forth herein.

According to yet additional aspect of the present invention there is provided a method for treating an animal for inducing protective immunity

against multiple sclerosis. The method is effected by administering, to the animal, an antibody capable of *in vivo* neutralizing an interferon gamma inducing factor in affecting cells to produce interferon gamma.

5 According to still additional aspect of the present invention there is provided a method for treating an animal for inducing protective immunity against multiple sclerosis. The method is effected administering, to the animal, an antigen including an interferon gamma inducing factor or an immunogenic portion thereof, thereby eliciting an antibody being capable of binding *in vivo* an interferon gamma inducing factor.

10 As used herein the term "antigen" refers to an immunogen including at least one immunogenic epitope, which is represented in the equivalent native peptide in a continuous or discontinuous fashion.

According to a preferred embodiment of the present invention, the antibody is polyclonal. Preparation of polyclonal antibodies is known in the art and further described in the Examples section hereinunder.

According to another preferred embodiment of the present invention, the antibody is monoclonal. Methods of producing and identifying monoclonal antibodies are well known in the art.

20 Monoclonal antibodies may be obtained by processes comprising the generation of a plurality of monoclonal antibodies to an antigen and screening the plurality of antibodies so generated to identify a monoclonal antibody that binds and/or neutralizes the peptide of interest, interferon gamma inducing factor in the present case. Monoclonal antibodies may be generated either *in vitro* or *in vivo*. In a related process, an animal is immunized with an antigen thereby generating antibody producing lymphocytes in said animal, antibody producing lymphocytes are removed from the animal, said lymphocytes are fused with myeloma cells to produce a plurality of immortalized hybridoma cells each of which produces monoclonal antibodies, the plurality of monoclonal antibodies is screened to identify a monoclonal antibody that binds the peptide, and the hybridoma producing the antibody is cloned and propagated. Animals are typically immunized with a mixture comprising a solution of the immunogen in a physiologically acceptable vehicle, and any suitable adjuvant, which achieves an enhanced immune response to the immunogen. By way of example, the primary immunization conveniently may be accomplished with a mixture of a solution of the immunogen and Freund's complete adjuvant, said mixture being prepared in the form of a water in oil emulsion. Typically the immunization may be administered to the animals

intramuscularly, intradermally, subcutaneously, intraperitoneally, into the footpads, or by any appropriate route of administration. The immunization schedule of the immunogen may be adapted as required, but customarily involves several subsequent or secondary immunizations using a milder adjuvant such as Freund's incomplete adjuvant. Antibody titers and specificity of binding to the hapten can be determined during the immunization schedule by any convenient method including by way of example radioimmunoassay, or enzyme linked immunoassay. Antibody activity assays can be performed in vitro as further exemplified in the Examples section that follows. When suitable antibody titers are achieved, antibody producing lymphocytes from the immunized animals are obtained, and these are cultured, selected and cloned, as is known in the art. Typically, lymphocytes may be obtained in large numbers from the spleens of immunized animals, but they may also be retrieved from the circulation, the lymph nodes or other lymphoid organs. Lymphocytes are then fused with any suitable myeloma cell line, to yield hybridomas, as is well known in the art. Alternatively, lymphocytes may also be stimulated to grow in culture, and may be immortalized by methods known in the art including the exposure of these lymphocytes to a virus, a chemical or a nucleic acid such as an oncogene, according to established protocols. After fusion, the hybridomas are cultured under suitable culture conditions, for example in multiwell plates, and the culture supernatants are screened to identify cultures containing antibodies that recognize the hapten of choice. Hybridomas that secrete antibodies that recognize the hapten of choice are cloned by limiting dilution and expanded, under appropriate culture conditions. Monoclonal antibodies are purified and characterized in terms of immunoglobulin type binding affinity and in vivo or in vitro neutralizing activity.

The antibody according to the present invention, be it a poly- or monoclonal antibody, is a neutralizing antibody to interferon gamma inducing factor in affecting cells to produce interferon gamma, that is to say that the antibody interferes with the functionality of interferon gamma inducing factor in affecting cells to produce interferon gamma.

According to an additional aspect of the present invention there is provided a method for treating an animal for inducing protective immunity against multiple sclerosis. The method according to the present invention is effected by administering, to the animal, cells capable of producing and secreting an antibody capable of *in vivo* neutralizing an interferon gamma

inducing factor in affecting cells to produce interferon gamma. To this end, cloned cDNAs encoding antibodies or fragments thereof are used to genetically modify receptive cells, to thereby render the cells antibody producing cells.

5        Cloning of cDNAs encoding antibodies or fragments thereof may be accomplished by several approaches known in the art. In the preferred approach, mRNA from clonal hybridoma cell lines which produce antibodies is employed as starting material. The cells are harvested and mRNA is extracted by standard methods known in the art. The cDNA is prepared by reverse transcription of the mRNA by standard methods known  
10        in the art. The cDNA for each chain of the immunoglobulin is cloned separately, and may be amplified by polymerase chain reaction using appropriate primers. The cDNA is then ligated into appropriate vectors by standard methods. The cDNA may be cloned into expression vectors and  
15        expressed separately in any convenient expression system, so that the properties of the expressed single chains of the antibodies may be determined. Alternatively, the individual chains may be expressed in the same cells which are then screened for the production of recombinant active antibodies. The method of using the invention will be modified in  
20        accordance with the system that is selected according to the current principles that are known in the art of recombinant protein production. According to the present invention the genetic information for the production of the antibody of interest is introduced into the cells by an appropriate vector as is known in the art or by any other acceptable means.  
25        The present invention provides information that will enable the skilled artisan to prepare constructs of genetic material comprising an open reading frame that encodes at least one chain of a novel antibody capable of binding IGIF. It will be appreciated that in certain embodiments it will suffice to produce an active fragment of the catalytic activity, for instance a Fab  
30        fragment of the intact antibody or even an Fv fragment thereof. In addition to the nucleic acid encoding the protein or polypeptide of choice, the constructs of the invention may comprise the following elements: a selectable marker, an origin of replication, a transcriptional promoter, a translation start site, a signal sequence for secretion of the product.

35        U.S. Pat. application No. 09/123,485, filed July 28, 1998, which is incorporated by reference as if fully set forth herein, teaches the effectiveness of DNA vaccines in inducing protective immunity against multiple sclerosis. In that application, DNA sequences encoding for a

variety of chemokines and for the cytokine tumor necrosis factor alpha, were shown to elicit protective immunity against both induced and transferred EAE, while the prior art teaches protective immunity against both induced and transferred EAE via passive vaccination (administration  
5 of antibodies).

Similarly, DNA vaccination in various forms, some of which are further detailed hereinunder, can be used to confer protective immunity against multiple sclerosis.

Thus, according to another aspect of the present invention there is provided a method for treating an animal for inducing protective immunity  
10 against multiple sclerosis. The method is effected by administering to the animal a therapeutic composition including a recombinant construct including an isolated nucleic acid sequence encoding a polypeptide being capable of eliciting antibodies capable of *in vivo* neutralizing an interferon gamma inducing factor in affecting cells to produce interferon gamma.  
15

According to yet another aspect of the present invention there is provided a method for treating an animal for inducing protective immunity against multiple sclerosis. The method is effected by implementing the following method steps, in which, in a first step, cells are removed from the  
20 animal. In a second step, the cells are genetically modified *in vitro* with a recombinant construct including an isolated nucleic acid sequence encoding an interferon gamma inducing factor or an immunogenic portion thereof. Finally, the genetically modified cells are reintroduced to the animal.

As used herein in the specification and in the claims section below,  
25 the term "genetically modified" refers to a process of inserting nucleic acids into cells. The insertion may, for example, be effected by transformation, viral infection, injection, transfection, gene bombardment, electroporation or any other means effective in introducing nucleic acids into cells. Following the modification the nucleic acid is either integrated in all or part,  
30 to the cell's genome (DNA), or remains external to the cell's genome, thereby providing stably modified or transiently modified cells. The cells according to this method of the invention may be of any kind. Especially suitable cells are those readily removable, genetically modifiable, and reintroduceable cells, such as, but not limited to, cells of the various blood  
35 lineages, derived either from whole blood or from bone marrow, fibroblast cells, etc. The genetically modified cells are preferably reintroduced to the animal parenterally.

According to still another aspect of the present invention there is provided a pharmaceutical composition for inducing protective immunity against multiple sclerosis. The composition comprises a pharmaceutically acceptable carrier and a recombinant construct including an isolated nucleic acid sequence encoding a polypeptide being capable of eliciting antibodies capable of *in vivo* neutralizing an interferon gamma inducing factor in affecting cells to produce interferon gamma.

According to a preferred embodiment of the present invention, the nucleic acid sequence is operatively linked to one or more transcription control sequences, such as, but not limited to, RSV control sequences, CMV control sequences, retroviral LTR sequences, SV-40 control sequences and/or  $\beta$ -actin control sequences. Preferably, the recombinant construct is an eukaryotic expression vector, such as, but not limited to, pcDNA3, pcDNA3.1(+/-), pZeoSV2(+/-), pSecTag2, pDisplay, pEF/myc/cyto, pCMV/myc/cyto, pCR3.1, pCI, pBK-RSV, pBK-CMV, pTRES, and their derivatives.

According to a preferred embodiment of the present invention, the therapeutic composition is administered to the animal parenterally. According to another preferred embodiment of the present invention, the animal is a human being.

According to still further features in the described preferred embodiments the pharmaceutically acceptable carrier is selected from the group consisting of an aqueous physiologically balanced solution, an artificial lipid-containing substrate, a natural lipid-containing substrate, an oil, an ester, a glycol, a virus and metal particles. Preferably, the pharmaceutically acceptable carrier comprises a delivery vehicle that delivers the nucleic acid sequences to the animal. The delivery vehicle can be effected by liposomes, micelles or cells.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

## EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

5

### *Materials and Experimental Methods*

**Rats:** Female Lewis rats, six weeks old, were purchased from Harlan (Israel) and maintained under SPF conditions in an animal facility.

**Peptide antigens:** Myelin Basic Protein ( MBP) p68-86, Y G S L P Q K S Q R S Q D E N P V (SEQ ID NO:1), was synthesized on a MilliGen 9050 peptide synthesizer by standard 9-fluorenylmethoxycarbonyl chemistry. Peptides were purified by high performance liquid chromatography. Structure was confirmed by amino acid analysis and mass spectroscopy. Only peptides that were greater than 95 % pure were used in our study.

**Immunizations and induction of active disease:** Rats were immunized subcutaneously in the hind foot pads with 0.1 ml of MBP epitope 68-86 (p68-86) dissolved in PBS (1.5 mg/ml) and emulsified with an equal volume of CFA (incomplete Freund's adjuvant supplemented with 4 mg/ml heat-killed *Mycobacterium tuberculosis* H37Ra in oil (Difco laboratories, Inc., Detroit, MI). Rats were then monitored for clinical signs daily by an observer blind to the treatment protocol. EAE was scored as follows: 0, clinically normal; 1, flaccid tail; 2, hind limb paralysis; 3, front and hind limb paralysis.

**Induction of transferred EAE:** EAE was induced by immunizing Lewis rats (intraperitoneally) with  $10^8$  activated spleen cells from EAE donors obtained as follows: Nine days after induction of active EAE, splenic cells were cultured ( $12 \times 10^6$ /ml) at 37 °C in humidified air containing 7.5 % CO<sub>2</sub> for two days in stimulation medium that includes Dulbecco's modified Eagle's medium (Gibco) supplemented with 2-mercaptoethanol ( $5 \times 10^{-5}$  M), L-glutamine (2 mM), sodium pyruvate (1 mM), penicillin (100 µg/ml), streptomycin (100 µg/ml), 1 % syngeneic serum and 20-30 µg/ml of the immunizing epitope. Then, cells were separated on a Ficoll gradient (Sigma), resuspended in PBS and injected to naive recipients.

**Antigen-specific T cell proliferation assays:** Lewis rats were immunized with MBP p68-86/CFA as described above. Nine to ten days later spleen cells were suspended in stimulation medium and cultured in U-

shape 96-well microculture plates ( $2 \times 10^5$  cells/well) for 72 hours, at 37 °C in humidified air containing 7.5 % CO<sub>2</sub>. Each well was pulsed with 2 µCi of [<sup>3</sup>H]-Thymidine (specific activity 10 Ci/mmol) for the final six hours. The cultures were then harvested on fiberglass filters and the proliferative response expressed as CPM ± S.E. or as stimulation index (SI) (mean CPM of test cultures divided by mean CPM of control cultures).

**Reverse transcriptase polymerase chain reaction (RT-PCR) analysis:** RT-PCR analysis, verified by Southern blotting, was utilized on brain samples according to the protocol described elsewhere with some modifications (39). Rats were euthanized by CO<sub>2</sub> narcosis. Brain samples containing mainly the midbrain and brain stem were obtained after perfusion of the rat with 160-180 ml of ice-cold phosphate buffered saline (PBS) injected into the left ventricle following an incision in the right atrium. Each sample was homogenized. Total RNA was extracted using the Tri-Zol procedure (Gibco BRL) according to the manufacturer's protocol. mRNA was then isolated using a mRNA isolation kit (#1741985, Boheringer Mannheim, Germany), and reverse transcribed into first strand cDNA as described in detail elsewhere (39). First strand cDNA was then subjected to 35 cycles of PCR amplification using specific oligonucleotide primers to rat IGIF and IFN-γ which were designed based on the published sequence of each cytokine (NCBI accession number for rat IGIF - U77777; and for rat IFN-γ - M29315 ) as follows (Table 1):

**TABLE 1**

Name/Function	Sequence	SEQ ID NO:
Rat IGIF sense	5'-ATGGCTGCCATGTCAGAAGAAG-3'	2
Rat IGIF antisense	5'-CTAACTTTGATGTAAGTTAGTAAGA-3'	3
Rat IFN-γ sense	5'-TACTGCCAAGGCACACTCATTGAA-3'	4
Rat IFN-γ antisense	5'-CGCTTCCTTAGGCTAGATTCTGG-3'	5
Rat β-actin sense	5'-CATCGTGGGCCGCTCTAGGCA-3' *	6
Rat β-actin antisense	5'-CCGGCCAGCCAAGTCCAGACG-3' *	7

Sequence according to Reference 39.

Experimental conditions were calibrated so RT-PCR amplifications fall on the linear part of the titration curve. The cycle profile was: denaturation at 95 °C for 60 seconds, annealing at 55 °C for 60 seconds, and



elongation at 72 °C for 60 seconds. Amplified products were subjected to electrophoresis, transferred to a nylon membranes (MagnaGraph nylon transfer membrane, msi, Westborough, MA), fixed with ultraviolet light (120 mJoules) and hybridized with 10<sup>6</sup> cpm/ml of a <sup>32</sup>P labeled DNA fragments encoding the full length PCR product of IGIF and of β-actin (random priming: Amersham, Arlington Heights, IL). PCR products were used as probes only after each PCR product was cloned and its sequence was verified as described below. Southern blot images were objectively assessed using an FujiFilm Thermal System FTI-500 (FujiFilm, Japan).

**Cloning and sequencing of PCR products:** Each of the amplified PCR products described above was cloned into a pUC57/T vector (T-cloning Kit #K1212, MBI Fermentas, Lithuania) and transformed to *E. coli* according to the manufacturer's protocol. Each clone was then sequenced (Sequenase version 2, USB, Cleveland, Ohio) according to the manufacturer's protocol.

**Production and purification of recombinant proteins.** After sequence verification, PCR products were recloned into a PQE expression vector (PQE-30, PQE-31 or PQE-32 according the correct open reading frame) and expressed in *E. Coli* (Qaigen, Hilden, GmbH) and then purified by an NI-NTA-super flow affinity purification of 6 x His proteins (Qaigen). Each recombinant protein sequence has been verified (N -terminus).

**Production and purification of Rabbit anti-rat IGIF IgG:** Rabbit anti-rat IGIF antibodies were generated as described (40) and IgG fraction was purified using a HiTrap protein G kit (Pharmacia, Piscataway, NJ, Kit #17-040-01). Antibody titer was determined by a direct ELISA assay: ELISA plates (Nunc, Denmark) were coated with recombinant rat IGIF(50 ng/well). Rabbit anti-rat IGIF (IgG fraction) was added in serial dilutions from 2<sup>8</sup> to 2<sup>30</sup>. Goat anti-rabbit IgG alkaline phosphatase conjugated antibodies (Sigma) were used as a labeled antibody. p-Nitrophenyl Phosphate(p-NPP) (Sigma) was used as a soluble alkaline phosphatase substrate. Results of triplicates were calculated as log 2 antibody titer ± SE. The purified anti-rat IGIF IgG titer was 18±0.4.

**Cytokine determination:** Spleen cells from EAE donors were stimulated *in vitro* (10<sup>7</sup> cells/ml) in 24 well plates (Nunc) with 100 μM p68-86. Spleen cells from naive donors were cultured (10<sup>7</sup> cells/ml, 24 well plates) with 2 μg/ml Con A (Sigma). After 72 hours of stimulation, supernatants were assayed by semi-ELISA kits, that include antibody pairs and recombinant rat cytokines, as follows: IFN-γ, rabbit anti-rat IFN-γ

polyclonal antibody (CY-048, Innogenetics, Belgium) as a capture antibody, biotinylated mouse anti-rat monoclonal antibody (CY-106 clone BD-1, Innogenetics) as a detection antibody, and Alkaline phosphatase-Streptavidin (cat No. 43-4322, Zymed, SF, CA) with rat recombinant IFN- $\gamma$  as a standard (Cat No 3281SA, Gibco BRL); TNF- $\alpha$ , commercial semi-ELISA kit for the detection of rat TNF- $\alpha$ , (Cat No 80-3807-00, Genzyme, Cambridge, MA); IL-4, mouse anti-rat IL-4 monoclonal antibody (24050D OX-81, PharMingen, San Diego, CA) as a capture antibody, and rabbit anti-rat IL-4 biotin-conjugated polyclonal antibody (2411-2D, PharMingen) as second antibody. Recombinant rat IL-4 purchased from R&D (504-RL) was used as a standard.

**Statistical analysis:** Significance of differences was examined using Student's t-test. Mann-Whitney sum of ranks test was used to evaluate significance of differences in mean of maximal clinical score (Figure 3). Value of  $p < 0.05$  was considered significant.

### Experimental Results

**IGIF mRNA is transcribed in the inflamed EAE brain:** Midbrain-brain stem samples were obtained from rats with developing transferred EAE (Figure 1A) before adoptive transfer of disease (day 0), and at various time points: before the onset of disease (day 3), at the day of onset (day 5), the peak (day 7), following recovery (day 10), and 10 days after recovery (day 20). For each time point, samples from six different brains were subjected to RT-PCR analysis using specific oligonucleotide primers which constructed to IGIF and IFN- $\gamma$ . Each amplification was calibrated to  $\beta$ -actin and verified by Southern blot analysis. This enabled semi-quantitative analysis of the dynamics of mRNA transcription of IGIF and IFN- $\gamma$  at the site of inflammation. Figures 1C and 1E show representative results from each time point of the experiment. A substantial increase in the transcription of both IGIF and IFN- $\gamma$  mRNA in EAE brains was observed at the peak of disease (day 7). The augmented transcription of IFN- $\gamma$  mRNA reverted to background levels during recovery. Unexpectedly, a notable transcription of IGIF mRNA could be observed even ten days after recovery (Figure 1C).

Rats with developing active disease manifested similar mRNA transcription characteristics as those with developing transferred disease. That is, a substantial increase in the transcription of both IGIF and IFN- $\gamma$  mRNA in EAE brains was observed at the peak of disease (day 13). The

augmented transcription of IFN- $\gamma$ , but not of IGIF mRNA, regressed to background level during recovery (Figures 1D and 1F). A substantial increase in the level of a IGIF transcription at the site of inflammation in the CNS during the course of disease may suggest involvement in its regulation.

5 To evaluate this point, the role of IGIF in regulation of EAE was investigated.

***Recombinant rat IGIF and its neutralizing antibodies affect IFN- $\gamma$  production by activated T cells from naive donors more significantly than by antigen specific primed T cells:*** PCR products encoding rat IGIF were  
10 used to generate the recombinant protein which was used to produce anti-IGIF neutralizing antibodies. These antibodies significantly reduced the production of IFN- $\gamma$  in primed T cells proliferating in response to their specific myelin basic protein (MBP) epitope (Figure 2C  $3.2 \pm 0.25$  versus  $1.8 \pm 0.11$  ng/ml with backgrounds of  $0.2 \pm 0.1$  and  $0.25 \pm 0.1$ ,  $p < 0.01$ )  
15 and entirely blocked IFN- $\gamma$  production in Con A activated T cells from naive donors (Figure 2A,  $5.1 \pm 0.4$  versus  $0.4 \pm 0.1$  ng/ml with backgrounds of  $0.4 \pm 0.1$  and  $0.36$ ,  $p < 0.001$ ). Control IgG from normal rabbit serum did not exert a notable effect on IFN- $\gamma$  production by either Con A activated naive spleen cells or MBP p68-86 primed spleen cells (data  
20 not shown). Recombinant rat IGIF elicited IFN- $\gamma$  production in Con A activated splenic T cells from naive donors (Figure 2B,  $15.8 \pm 0.8$  ng/ml versus  $5.1 \pm 0.3$  with backgrounds of  $0.3 \pm 0.1$  and  $0.4 \pm 0.15$ ,  $p < 0.001$ ) and significantly, though again less profoundly, the response of primed spleen T cells to their target MBP antigen (Figure 2D,  $4.97 \pm 0.15$  ng/ml  
25 versus  $3.2 \pm 0.25$ , with backgrounds of  $0.3 \pm 0.15$  and  $0.25 \pm 0.1$ ,  $p < 0.001$ ). Thus, both the inhibitory effect of IGIF neutralizing antibodies and the augmentation by IGIF of IFN- $\gamma$  production are more profound on activated T cells from a naive donor than on primed T cells responding to their target epitope. It has recently been suggested that IGIF primarily  
30 affects IFN- $\gamma$  production by Th1 not Th2 cells (29). It is possible that immunization with p68-86/CFA induces a substantial selection of antigen specific Th2 cells, albeit not enough to inhibit the subsequent development of a Th1 mediated autoimmune disease.

The *in vitro* addition of either anti-IGIF antibodies or of recombinant  
35 IGIF did not affect the antigen specific proliferative response developed in primed splenic T cells responding to MBP p68-86 (SI =  $4.2 \pm 0.3$ ,  $3.6 \pm 0.4$  and  $3.9 \pm 0.3$  in control spleen T cells) versus cultured spleen cells supplemented with either anti-IGIF antibodies or recombinant IGIF.

***Neutralizing antibodies to recombinant rat IGIF block the development of both active and transferred EAE:***

The role of anti-IGIF antibodies in the regulation of T cell mediated autoimmune diseases has never been explored before. Herein the competence of the anti-IGIF neutralizing antibodies to inhibit active (Figure 3A) and transferred (Figure 3B) EAE is evaluated. Lewis rats were immunized with p68-86/CFA to develop active EAE. Just before the onset of disease (days eight and ten) and at the onset of disease (day eleven) these rats were injected with either rabbit anti-rat IGIF (IgG fraction), IgG fraction purified from non-immunized rabbits (control IgG), or with PBS, and monitored for clinical signs of EAE. Control PBS treated rats and rats treated with control IgG all (6/6 rats in each group) developed severe EAE (Mean maximal clinical score  $3.3 \pm 0.43$  and  $2.66 \pm 0.26$ , respectively). In contrast, rats treated with anti-IGIF antibodies developed a markedly reduced disease (Figure 3A, incidence 5/6, mean maximal clinical score  $1.2 \pm 0.2$ ,  $p < 0.01$ ).

The competence of anti-IGIF antibodies to inhibit transferred EAE (Figure 3B) was further evaluated. Three, five and seven days after adoptive transfer of disease rats were injected as described above and monitored for clinical signs of EAE. While control PBS treated rats and rats treated with control IgG have all (6/6 rats in each group) developed EAE (mean maximal clinical score  $1 \pm 0$  in each group) rats administered with anti-IGIF antibodies were highly protected (Figure 3B, incidence 1/6, mean maximal clinical score  $0.2 \pm 0.1$ ,  $p < 0.01$ ). Thus, immunotherapy with anti-IGIF serves as a powerful tool to block the development of actively induced or transferred EAE.

***Alteration of IFN- $\gamma$  and IL-4 production in EAE rats injected with anti-IGIF neutralizing antibodies suggests that perturbation of the Th2/Th1 balance contributes to disease blockade:***

The possible involvement of a Th2/Th1 switch in EAE inhibition by anti-IGIF immunotherapy has been evaluated (Figure 4). Lewis rats were immunized with p68-86/CFA to develop active EAE. Five and seven days later these rats were injected with either PBS, control rabbit IgG or rabbit anti-rat IGIF (IgG fraction). Two days after the last treatment, splenic T cells were cultured with MBP p68-86 in stimulation medium that was (Figures 4C and 4D) or was not supplemented with recombinant rat IL-4 (Figures 4A and 4B). In spleen cells cultured from MBP 68-86 primed donors, IFN- $\gamma$  was produced only when the priming antigen was added to the culture (Figures 4A,  $0.3 \pm 0.1$  ng/ml without addition of MBP 68-86 versus  $13.5 \pm 0.7$  in

cells proliferating to p68-86). Addition of recombinant IL-4 led to a significant decrease in IFN- $\gamma$  which was still dependent upon antigenic stimulation (Figures 4A and 4C  $0.19 \pm 0.08$  ng/ml without addition of MBP 68-86 versus  $2.37 \pm 0.8$  in cells proliferating to p68-86, a 12 fold increase).

5 Spleen T cells from anti-IGIF treated rats produced markedly reduced levels of IFN- $\gamma$  in response to antigenic stimulation in cultures that were or were not supplemented with IL-4 (Figure 4A,  $4.7 \pm 0.4$  ng/ml in spleen cells from anti-IGIF treated rat versus  $9.7 \pm 0.8$  in spleen cells from rats treated with normal rabbit IgG and  $13.5 \pm 0.7$  in PBS treated rats, with backgrounds of

10 0.4, 0.8 and 0.7,  $p < 0.001$ , when comparing anti-IGIF treatment to each control group). IL-4 production, however, markedly increased in splenic T cells from anti-IGIF treated rats regardless of *in vitro* stimulation (Figure 4B,  $62.3 \pm 4.2$  pg/ml in spleen cells from anti-IGIF treated rat versus  $15.3 \pm 0.4$  in spleen cells from rats treated with normal rabbit IgG and  $15.6 \pm 0.6$  in

15 PBS treated rats,  $p < 0.001$ , when comparing anti-IGIF treatment to each control group) unless cultures were supplemented with IL-4 (Figure 4D,  $1860 \pm 120$  pg/ml in spleen cells from anti-IGIF treated rat versus  $570 \pm 30$  in spleen cells from rats treated with normal rabbit IgG and  $450 \pm 35$  in PBS treated rats, with backgrounds of 85, 42 and 34,  $p < 0.0001$ , when

20 comparing anti-IGIF treatment to each control group). Addition of IL-4 to cultured spleen T cells (Figure 4C-D) did not exhibit a notable effect on their antigen specific proliferative response (data not shown).

TNF- $\alpha$  production was then evaluated in spleen cells from the above groups. The above spleen cells from anti-IGIF treated rats produced

25 markedly reduced levels of TNF- $\alpha$  in response to antigenic stimulation (Figure 5,  $850 \pm 45$  pg/ml in spleen cells from anti-IGIF treated rats versus  $1975 \pm 80$  in spleen cells from rats treated with normal rabbit IgG and  $2100 \pm 110$  in PBS treated rats, with backgrounds of 230, 210 and 270, respectively,  $p < 0.001$ , when comparing anti-IGIF treatment to each control

30 group). Thus perturbation of the Th1/Th2 balance in anti-IGIF treated rats is associated with a marked reduction in TNF- $\alpha$  production.

Finally, the proliferative response of each group of cultured spleen cells to p68-86 was evaluated in a proliferation assay. (SI =  $4.2 \pm 0.3$ ,  $3.6 \pm 0.4$  and  $3.96 \pm 0.5$  in spleen cells from rats treated with either anti-IGIF,

35 normal rabbit IgG or PBS respectively). Thus anti-IGIF immunotherapy alters Th2/Th1 balance without a notable affect on antigen specific proliferative responsiveness.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications  
5 and variations that fall within the spirit and broad scope of the appended claims.

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WHAT IS CLAIMED IS:

1. An antibody comprising an immunoglobulin capable of binding an interferon gamma inducing factor.
2. The antibody of claim 1, wherein the antibody is polyclonal.
3. The antibody of claim 1, wherein the antibody is monoclonal.
4. The antibody of claim 1, wherein said antibody is a neutralizing antibodies to said interferon gamma inducing factor in affecting cells to produce interferon gamma.
5. The antibody of claim 1, wherein the antibody is humanized.
6. A method for treating an animal for inducing protective immunity against multiple sclerosis, the method comprising the step of administering to said animal cells being capable of producing and secreting an antibody capable of neutralizing an interferon gamma inducing factor in affecting cells to produce interferon gamma.
7. The method of claim 6, wherein the antibody is humanized.
8. A method for treating an animal for inducing protective immunity against multiple sclerosis, the method comprising the step of administering to said animal an antibody capable of neutralizing an interferon gamma inducing factor in affecting cells to produce interferon gamma.
9. The method of claim 8, wherein the antibody is humanized.
10. A pharmaceutical composition for inducing protective immunity against multiple sclerosis, comprising a pharmaceutically acceptable carrier and an antibody being capable of binding an interferon gamma inducing factor.

11. The pharmaceutical composition of claim 10, wherein the antibody is humanized.

12. A method for treating an animal for inducing protective immunity against multiple sclerosis, the method comprising the step of administering to said animal an antigen including an interferon gamma inducing factor or an immunogenic portion thereof, thereby eliciting an antibody being capable of binding an interferon gamma inducing factor.

13. A pharmaceutical composition for inducing protective immunity against multiple sclerosis, comprising a pharmaceutically acceptable carrier and an interferon gamma inducing factor or an immunogenic portion thereof, thereby eliciting an antibody being capable of binding an interferon gamma inducing factor.

14. A method for treating an animal for inducing protective immunity against multiple sclerosis, the method comprising the step of administering to said animal a therapeutic composition including a recombinant construct including an isolated nucleic acid sequence encoding a polypeptide being capable of eliciting antibodies capable of neutralizing an interferon gamma inducing factor in affecting cells to produce interferon gamma.

15. The method of claim 14, wherein said nucleic acid sequence being operatively linked to one or more transcription control sequences.

16. The method of claim 15, wherein said transcription control sequences are selected from the group consisting of RSV control sequences, CMV control sequences, retroviral LTR sequences, SV-40 control sequences and  $\beta$ -actin control sequences.

17. The method of claim 14, wherein said recombinant construct is an eukaryotic expression vector.

18. The method of claim 14, wherein said recombinant construct is selected from the group consisting of pcDNA3, pcDNA3.1(+/-), pZeoSV2(+/-), pSecTag2, pDisplay, pEF/myc/cyto, pCMV/myc/cyto, pCR3.1, pCI, pBK-RSV, pBK-CMV, pTRES and their derivatives.

19. The method of claim 14, wherein said therapeutic composition is administered to the animal parenterally.

20. The method of claim 14, wherein said animal is a human being.

21. A method for treating an animal for inducing protective immunity against multiple sclerosis, the method comprising the steps of:

- (a) removing cells of said animal;
- (b) genetically modifying said cells in vitro with a recombinant construct including an isolated nucleic acid sequence encoding an interferon gamma inducing factor or an immunogenic portion thereof; and
- (c) reintroducing said genetically modified cells to said animal.

22. The method of claim 21, wherein said nucleic acid sequence is operatively linked to one or more transcription control sequences.

23. The method of claim 22, wherein said transcription control sequences are selected from the group consisting of RSV control sequences, CMV control sequences, retroviral LTR sequences, SV-40 control sequences and  $\beta$ -actin control sequences.

24. The method of claim 21, wherein said recombinant construct is an eukaryotic expression vector.

25. The method of claim 21, wherein said recombinant construct is selected from the group consisting of pcDNA3, pcDNA3.1(+/-), pZeoSV2(+/-), pSecTag2, pDisplay, pEF/myc/cyto, pCMV/myc/cyto, pCR3.1, pCI, pBK-RSV, pBK-CMV, pTRES and their derivatives.

26. The method of claim 21, wherein said genetically modified cells are reintroduced to the animal parenterally.

27. The method of claim 21, wherein said animal is a human.

28. A pharmaceutical composition for inducing protective immunity against multiple sclerosis, comprising a pharmaceutically

acceptable carrier and a recombinant construct including an isolated nucleic acid sequence encoding a polypeptide being capable of eliciting antibodies capable of neutralizing an interferon gamma inducing factor in affecting cells to produce interferon gamma.

29. The pharmaceutical composition of claim 28, wherein said pharmaceutically acceptable carrier is selected from the group consisting of an aqueous physiologically balanced solution, an artificial lipid-containing substrate, a natural lipid-containing substrate, an oil, an ester, a glycol, a virus and metal particles.

30. The pharmaceutical composition of claim 28, wherein said pharmaceutically acceptable carrier comprises a delivery vehicle that delivers said nucleic acid sequences to said animal.

31. The pharmaceutical composition of claim 30, wherein said delivery vehicle is selected from the group consisting of liposomes, micelles, and cells.

32. The pharmaceutical composition of claim 28, wherein said recombinant construct is an eukaryotic expression vector.

33. The pharmaceutical composition of claim 28, wherein said composition is suitable for parenteral administration to a human.

34. The use of an anti interferon gamma inducing factor antibody in the treatment of multiple sclerosis.



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**ABSTRACT OF THE DISCLOSURE**

**The use of anti interferon gamma inducing factor antibody in the treatment of multiple sclerosis.**

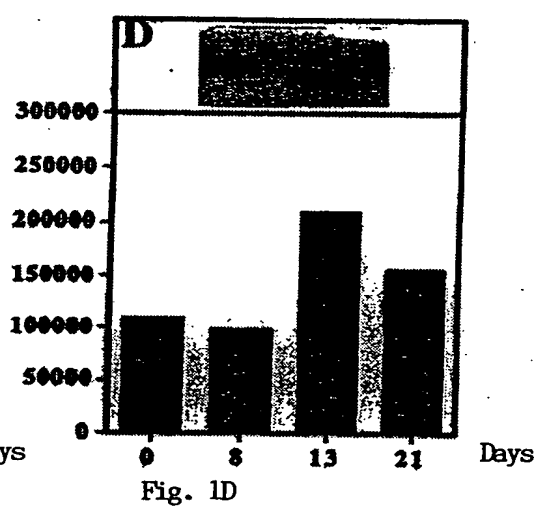
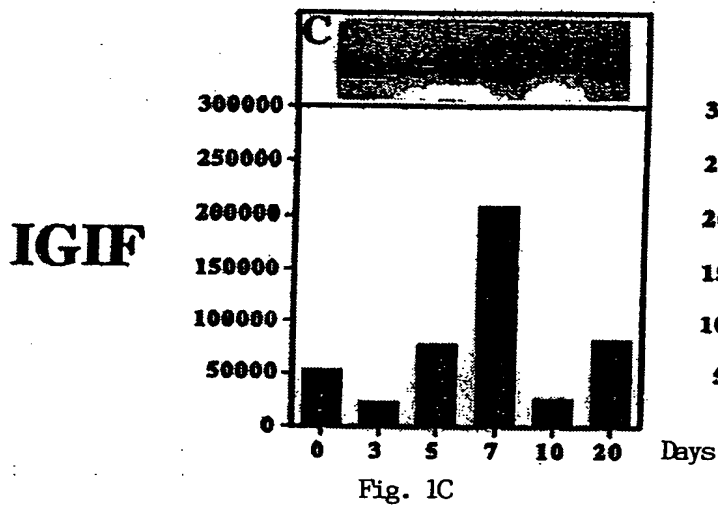
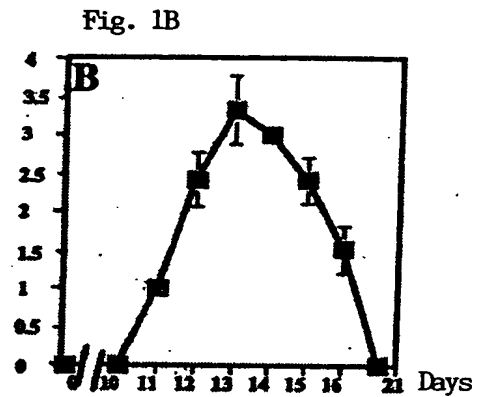
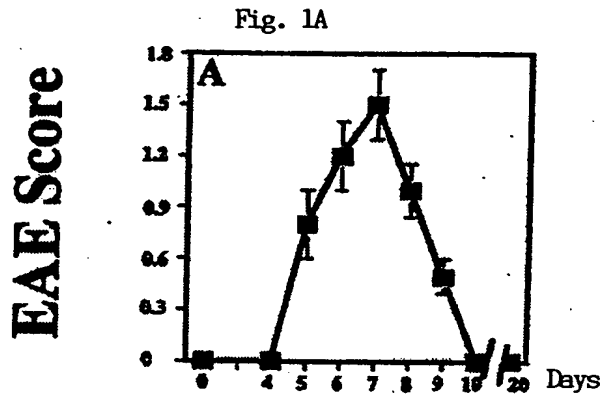


Fig. 1E

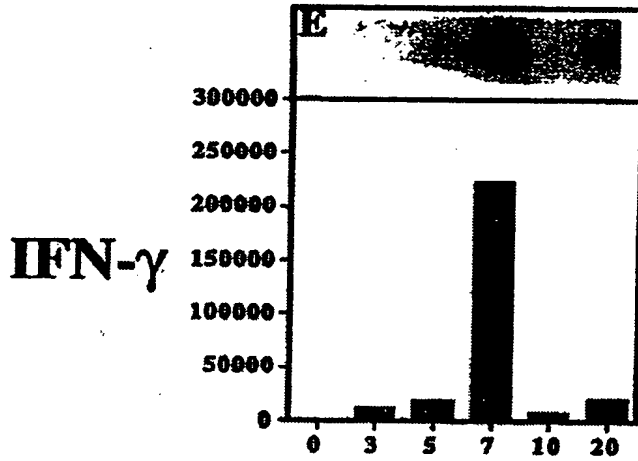


Fig. 1F

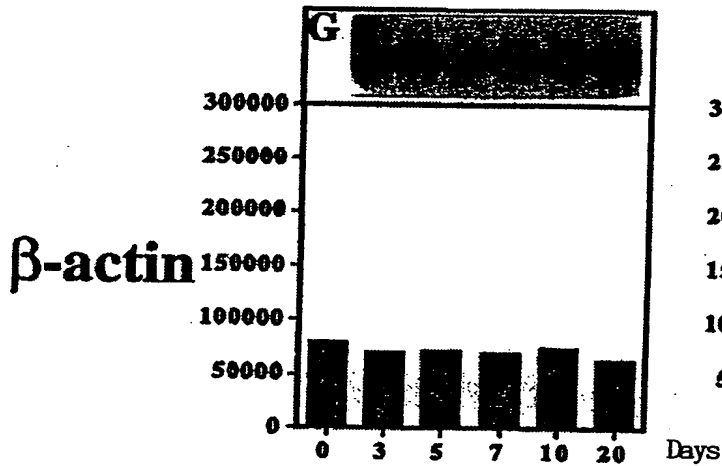
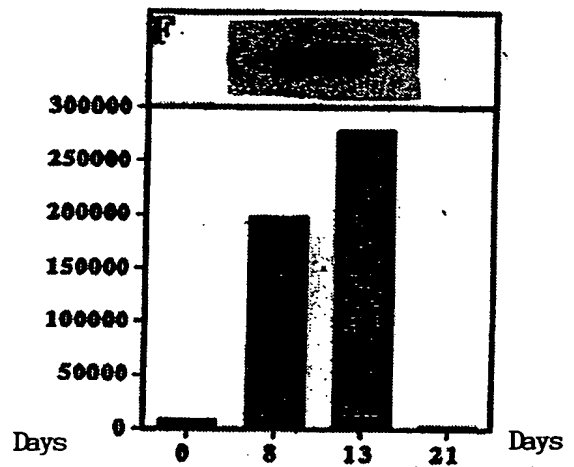


Fig. 1G

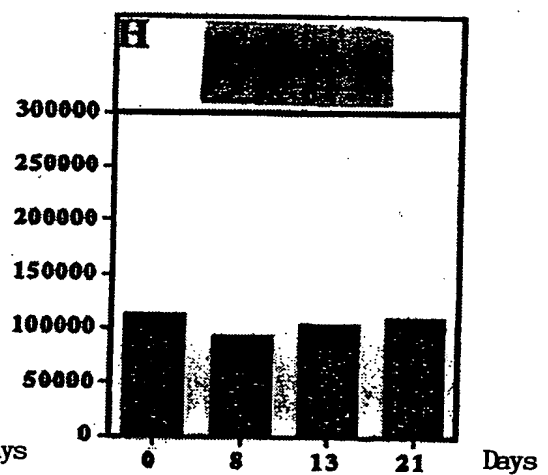


Fig. 1H

Fig. 2A

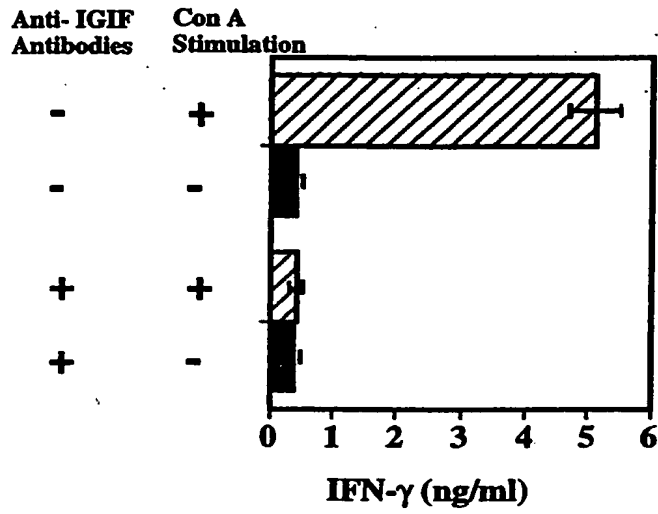


Fig. 2B

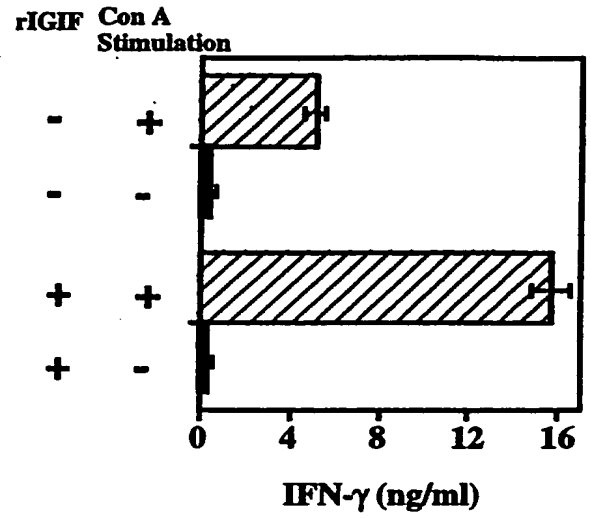


Fig. 2C

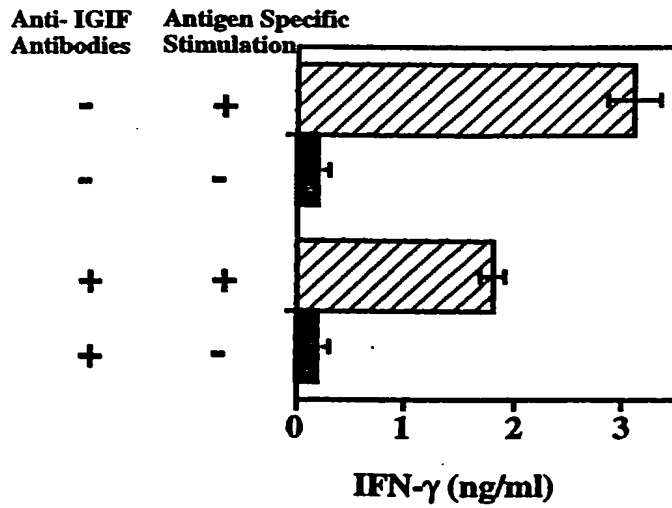
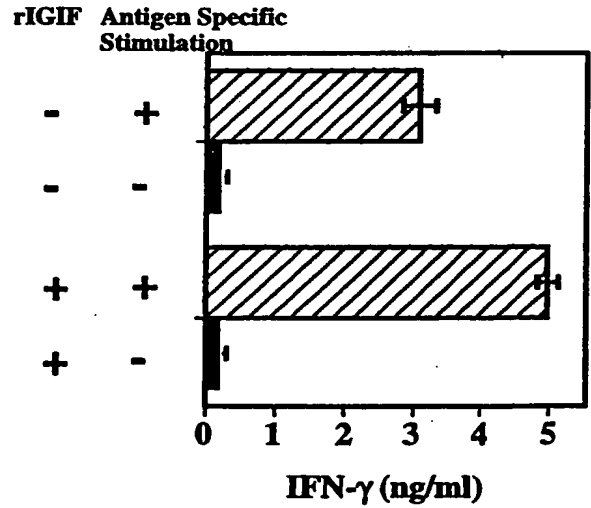
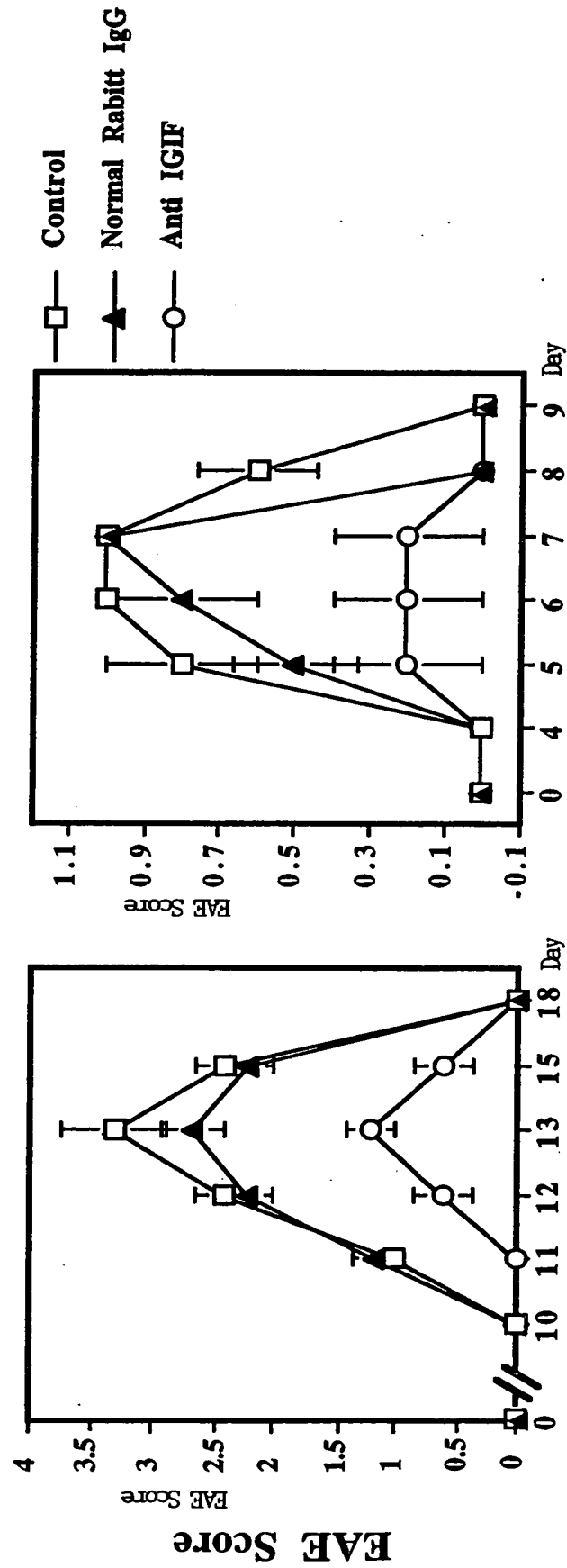
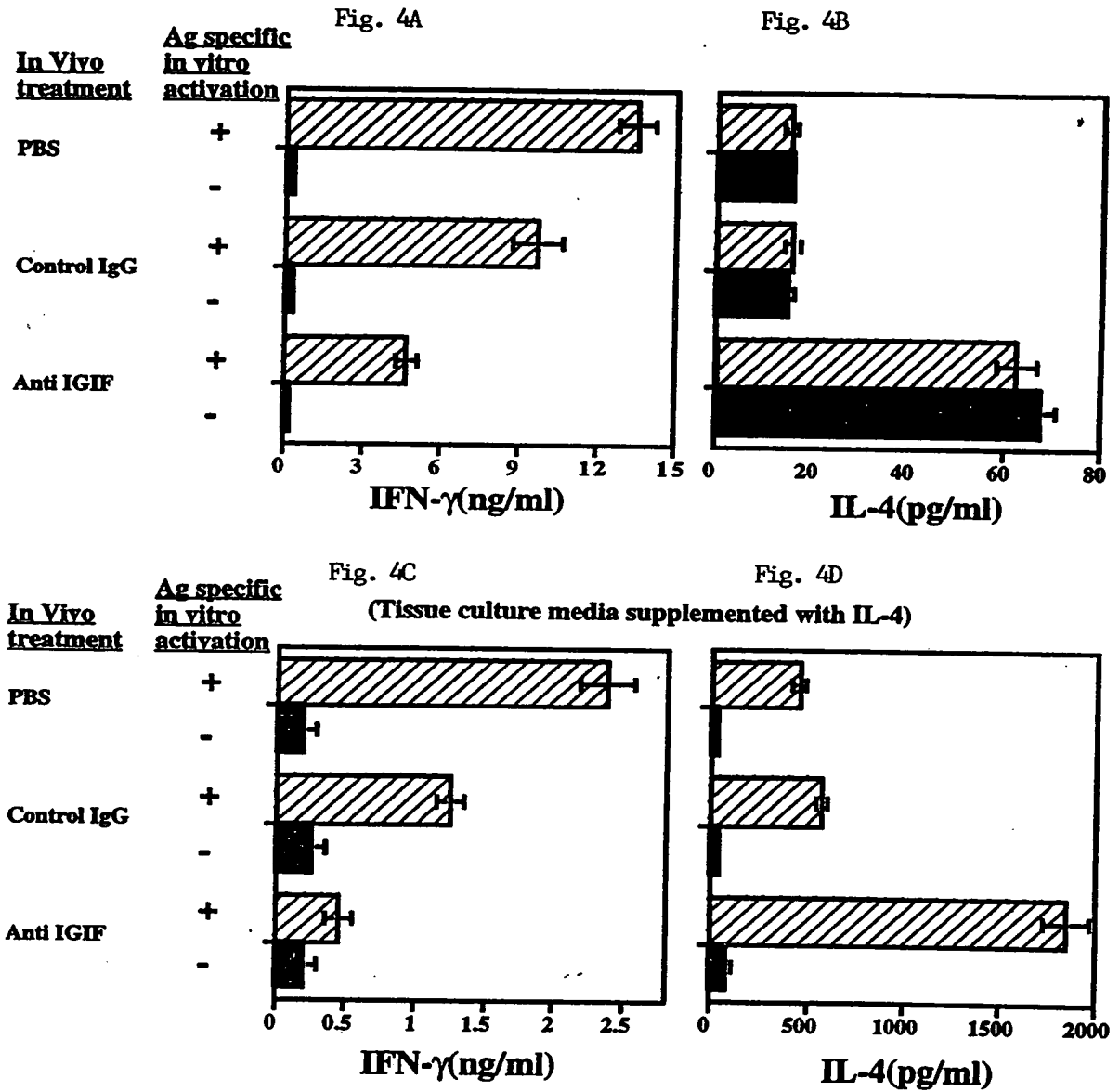


Fig. 2D

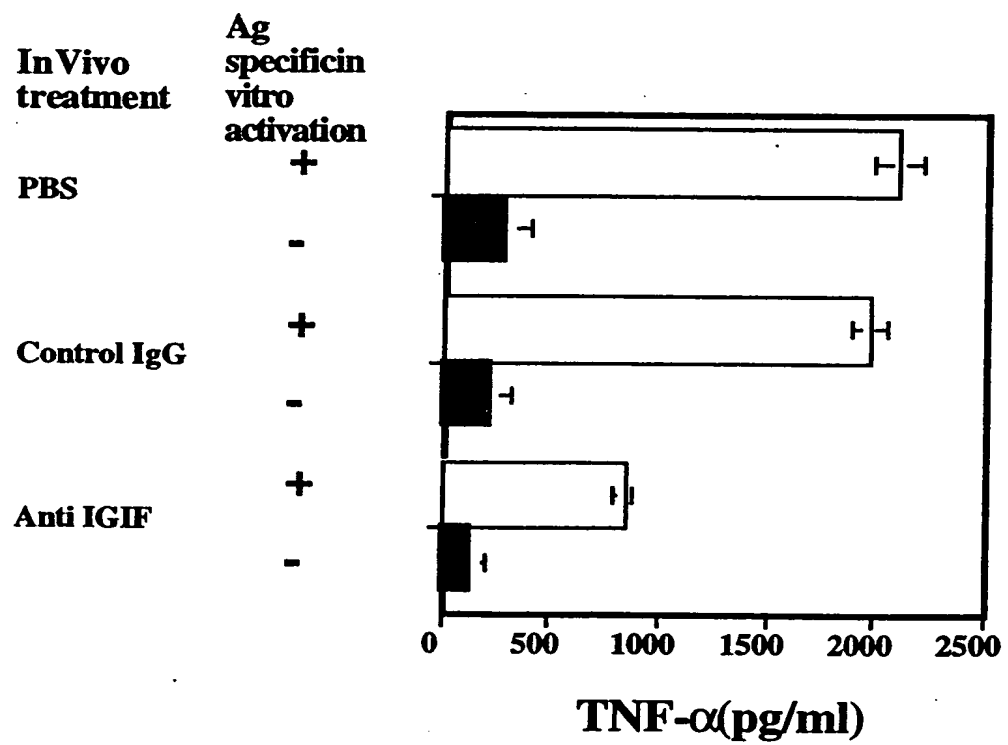




5/6



**Fig 5**



1

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANTS: Nathan Karin et al.
- (ii) TITLE OF INVENTION: INTERFERON GAMMA INDUCING FACTOR BASED  
VACCINE AND USE OF SAME FOR  
PROTECTIVE IMMUNITY AGAINST MULTIPLE  
SCLEROSIS
- (iii) NUMBER OF SEQUENCES: 7
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Mark M. Friedman c/o Anthony Castorina
  - (B) STREET: 2001 Jefferson Davis Highway, Suite 207
  - (C) CITY: Arlington
  - (D) STATE: Virginia
  - (E) COUNTRY: United States of America
  - (F) ZIP: 22202
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: 1.44 megabyte, 3.5" microdisk
  - (B) COMPUTER: Twinhead® Slimnote-890TX
  - (C) OPERATING SYSTEM: MS DOS version 6.2,  
Windows version 3.11
  - (D) SOFTWARE: Word for Windows version 2.0 converted  
to an ASCII file
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Friedmam, Mark M.
  - (B) REGISTRATION NUMBER: 33,883
  - (C) REFERENCE/DOCKET NUMBER: 910/5
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 972-3-5625553
  - (B) TELEFAX: 972-3-5625554
  - (C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 17
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Tyr Gly Ser Leu Pro Gln Lys Ser Gln Arg Ser Gln Asp Glu Asn  
                           5                  10                  15

Pro Val  
 17

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single



2

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:  
ATGGCTGCCA TGTCAGAAGA AG 22

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:  
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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:  
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(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:  
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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:  
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(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:  
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